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Special Issue Reprint

Improving Fertilizer Use Efficiency

Methods and Strategies for the Future

Edited by
Przemysław Barłóg, Jim Moir, Lukáš Hlisnikovský and Xinhua He

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About the Editors

Przemysław Barłóg

Dr. Przemysław Barłóg has been working at the Poznan University of Life Sciences in Poland for 30 years as an academic teacher and scientist. Currently, he works as a professor in the Department of Agricultural Chemistry and Environmental Biogeochemistry. Under his supervision, more than 100 students have achieved a Master of Sciences (Msc) degree and three students have achieved a PhD in the field of soil fertilization, plant nutrition, and environmental protection. Dr. Przemysław Barłóg is the author of more than 100 original, peer-reviewed scientific papers, most of which are indexed in world databases such as Scopus. He is also the author of numerous monographs and popular articles concerning the basis of crop plant nutrition and fertilization, addressed to students and advisory staff in agriculture.

Jim Moir

My research focus is soil fertility based, investigating nutrient cycling in grazed grasslands, including plant nutrition. My research expertise is in nitrogen (N) and phosphorus (P) cycling and soil pH (acidity) issues in soil/plant/animal systems. My aim is to improve the efficiency and effectiveness of our grassland farming systems, towards the future sustainability of grazed grasslands. In collaboration with colleagues at Qinghai University I am working on a key project examining nutrient flows and sustainability issues in high altitude Chinese and New Zealand grasslands. In China, we work on the Qinghai-Tibetan Plateau, which is under high grazing pressure from farmers and threatened by severe degradation as a result. This is the largest grassland Plateau in the world and shares many common issues with New Zealand high country grasslands. This project aims to increase knowledge of these systems to improve the quality and long-term sustainability of grazed grasslands.

Lukáš Hlisnikovský

Lukáš Hlisnikovský works as a plant nutrition scientist. His focus is on long-term experiments. In these, he studies how different fertilization methods, sowing practices, and weather affect the yield and quality of the crops grown. His interests also include the study of the effect of fertilization on soil chemistry.

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Xinhua He is currently Professor at Sichuan Agricultural University, China, Research Professor at UC Davis and Adjunct Professor at University of Western Australia (UWA). After his PhD study at University of Queensland/Australia in 2002, Xinhua has then worked as Postdoctoral Fellow at UC Davis, University of Tokyo, and UWA; Research Scientist at USDA's Forest Service; and Associate Professor at UWA and University of Sydney. Xinhua has been focusing on carbon and nitrogen cycling in plant-soil-microbe systems, soil beneficial microbes in plant/soil health and interplant C/N movement under global environmental change scenarios, by employing routine ecophysiological and microbial approaches, ^{13}C and ^{15}N stable isotopes, and high-throughput sequencing, etc.. He has been serving as editorial board/organizing committee members for diverse international peer-reviewed journals/conferences. At present Xinhua has >250 journal papers with 9,400 citations.

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Editorial

Improving Fertilizer Use Efficiency—Methods and Strategies for the Future

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Abstract: This editorial introduces our Special Issue entitled "Improving Fertilizer Use Efficiency—Methods and Strategies for the Future". The fertilizer use efficiency (FUE) is a measure of the potential of an applied fertilizer to increase the productivity and utilization of the nutrients present in the soil/plant system. FUE indices are mainly used to assess the effectiveness of nitrogen (N), phosphorus (P), and potassium (K) fertilization. This is due to the low efficiency of use of NPK fertilizers, their environmental side effects and also, in relation to P, limited natural resources. The FUE is the result of a series of interactions between the plant genotype and the environment, including both abiotic and biotic factors. A full recognition of these factors is the basis for proper fertilization in farming practice, aimed at maximizing the FUE. This Special Issue focuses on some key topics in crop fertilization. Due to specific goals, they can be grouped as follows: removing factors that limit the nutrient uptake of plants; improving and/or maintaining an adequate soil fertility; the precise determination of fertilizer doses and application dates; foliar application; the use of innovative fertilizers; and the adoption of efficient genotypes. The most important nutrient in crop production is N. Hence, most scientific research focuses on improving the nitrogen use efficiency (NUE). Obtaining high NUE values is possible, but only if the plants are well supplied with nitrogen-supporting nutrients. In this Special Issue, particular attention is paid to improving the plant supply with P and K.

Keywords: ammonia volatilization; controlled-release fertilizers; crop genotypes; elemental sulfur; magnesium; nitrogen use efficiency indices; phosphorus; potassium; root architecture; sustainability; Soil Fertility Clock

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1. Introduction—Why Fertilizer Use Efficiency Should Be Improved

According to forecasts, 9.7 billion people will be living on Earth by 2050, and about 10.4 billion by 2100 [1]. Right now, the world has the resources to feed a population of 8 billion. It is, therefore, necessary to seek optimal solutions in both the political and economic areas in order to solve the problem of the ever-growing demand for food. The expansion of agricultural areas at the expense of forests or shrubs, or even barren lands, either requires too much investment or is too risky in terms of the environment and the functioning of the global ecosystem [2–4]. Hence, the only rational direction for agricultural development is to maximize yields from the area already covered by agricultural activity [5,6]. There are some factors that are considered crucial in activities towards yield increase: breeding progress, the effective use of mineral fertilizers and crop protection measures, and farmers and their advisers having sufficient knowledge and skills [7]. The consumption of nitrogen fertilizers plays a special role in achieving this objective [8]. Mogollon et al. [9] presented several simulations showing that the global N input in agriculture in 2050 may fluctuate widely, ranging from 87 to 260 Tg N yr^{-1}. One of the main factors differentiating the above range is the nitrogen use efficiency (NUE). Currently, it is assumed that recovery of N from applied fertilizers is at the low level of just 30–50% [10]. As a result, N that is not taken up by plants is dispersed into the environment, reducing the economic profitability of agricultural production and, at the same time, causing a number of adverse changes in the functioning of the biosphere. The most important of these concern such phenomena as greenhouse gas and ammonia emissions, the destruction of the ozone layer, the eutrophication of the environment, or the impoverishment of ecosystems in plant and animal species [11]. An increase in the NUE value can be achieved through the improvement of N fertilization technology, including the use of innovative solutions in the production technology and chemical composition of fertilizers [12]. An important factor shaping the NUE is the presence of appropriate amounts and forms of minerals in the soil, which support the uptake and processing of N into plant crops [7]. This objective can be achieved using P, K, Mg, S, and other fertilizers, separately, or together with N in compound fertilizers. Hence, the term "NUE" can be extended to include the concept of fertilizer use efficiency (FUE). Such a definition allows for a broader approach to the issues related to the effectiveness of the application of all fertilizers and is not limited to only one nutrient. The aim of this Special Issue is to present the latest knowledge and research results regarding the improvement of the FUE/NUE in the cultivation of various plant species.

2. Special Issue Overview—General Topics

2.1. Factors Effecting Fertilizer Use Efficiency (FUE)

The first chapter of the Special Issue comprises two papers that focus on factors limiting the uptake and use of nutrients from fertilizers by crops, as well as on the present strategies and prospects for improving fertilizer use efficiency [12]. The term fertilizer use efficiency (FUE) is not new. It has been widely used for decades, but has become widespread thanks to the use of the FUE indices to assess the global productivity of NPK fertilizers. The number of indices used to characterize the FUE is vast, and their choice depends on the purpose of the analysis and/or comparisons [10,13]. The first article in the presented series of publications shows the concepts and principles of calculating a relatively new index, which is the nitrogen gap (NG). The NG calculation is important for the identification of hotspots in N management for a given crop, including the inadequate supply of nutrients other than N and a set of activities needed to improve the level of soil fertility for a given crop [14,15]. The impact of soil factors on the FUE should be considered as several groups of phenomena and processes [12]. The first group refers to factors affecting the nutrient uptake. However, there is a major challenge for a farmer to synchronize the crop plant requirement for nutrients with their supply from both soil and applied fertilizers. Achieving this goal requires extensive knowledge of plant growth dynamics and the critical phases of crop formation. After recognizing the nutritional requirements of plants, another area of activities aimed at improving the FUE is to create optimal conditions for plant root growth and eliminate all factors limiting the inflow of nutrients to the root surfaces. The most important soil factors shaping the uptake of nutrients and the FUE are the soil texture, water content, soil compaction, temperature, soil reaction, salinity, soil organic matter, and nutrient shortage [12]. Among them, the soil compaction and pH are relatively easy to correct in agricultural practices. The presented literature shows that FUE values can be shaped by building appropriate root architecture (RSA) in crops. This is possible by applying proper fertilization with N and K [16]. Another way to improve the FUE is the use of new and innovative fertilizers [12].

The second overview article presents the concept of effective fertilization, defined as the Soil Fertility Clock (SFC) [7]. At the core of this concept, there are three basic facts: (i) a crop plant in a well-defined geographic area, provided with stable environmental and nutritional conditions, can reach maximum yield (Y_{max}); (ii) the key production factor is N, present in the soil or/and supplied to the plant as fertilizer (organic and mineral, N_f); (iii) all other nutrients, called nitrogen-supporting nutrients (N-SNs), affect the Y_{max}, in relation to their relative deficiency in available form in the plant rooting zone. The classic concepts of N-SNs do not take into account that crop plants differ in their sensitivity to the supply of N-SNs in two crucial aspects: during the growing season and in the course of crop rotation. The Soil Fertility Clock (SFC) is an approach based on three assumptions: (i) the critical soil fertility is the value or range of soil nutrient content that is sufficient to provide an appropriate amount to the plant most sensitive to its supply in a given crop rotation; (ii) the non-sensitive plants in the given crop rotation create the necessary timeframe for the recovery of its original critical content; and (iii) the content of a specific nutrient cannot be a limiting factor in N uptake and utilization for any crop grown. The SFC concept is supported primarily by the yield-promoting role of P and K [7]. A deficiency of both nutrients in the soil during the critical stages of yield formation results in a decreased N_f efficiency, and consequently, a lower yield. Thus, the main goal of P and K application to the soil is to restore their content in the topsoil to the level required by the most sensitive crop in any given crop rotation.

2.2. Improving FUE by Optimizing N Uptake and Rate

One of the most important activities aimed at improving the FUE is the correct selection of the fertilizer dose for specific soil and climatic conditions, the applied agrotechnics, and the plants requirements in crop rotation. This can be achieved using analytical tools such as soil testing, plant tissue analysis, nutrient uptake dynamics, fertilizer rate response modeling, or digital and information technologies [7]. The standard methodology for determining the need for N fertilizers is based on data regarding the mineral nitrogen (N_{min}) content in the soil [17]. Therefore, it is extremely important to identify and classify the factors affecting the mineralization processes of organic nitrogen and the N_{min} content in the soil. The knowledge gained in this area can be translated into conscious control of N_{min}, thus shaping the yield level. The first article included in the subsection discusses the influence of various tillage practices on the content of different forms of N in fluvo-aquic soil from Huang-Huai-Hai Plain in China [18]. The experiment evaluated the effect of five treatments where rotary tillage (RT), deep tillage (DT), and shallow rotary tillage (SRT) were used. The test plant was wheat. The results showed that the rotation tillage with deep tillage increased the total N and the content of the mineral nitrogen forms compared with RT-RT-RT. They especially improved the NO_3–N and NH4–N content in 0–40 cm, with the highest value under DT-SRT-RT. However, the effect of deep tillage on dissolved organic N in deeper layers significantly declined with time. The highest wheat yield was under DT-SRT-RT in 2018 and 2019, with 6346 and 6557 kg ha^{-1}, respectively. The N partial productivity demonstrated a similar trend with the wheat yield, with higher values of 28.98 and 29.94 kg^{-1}, respectively. The authors also obtained the lowest apparent nitrogen loss values in the DT-SRT-RT treatment. It was suggested as the efficiency tillage practice to improve the NUE and the crop yield [18].

In field conditions, plants compete with each other for water and nutrients. Therefore, it is important to recognize the appropriate sowing density (SR) to minimize these effects and, at the same time, consciously combine yield components to obtain the maximum N productivity. The problem of the NUE's dependence on the sowing density in winter wheat cultivation in Jiangsu province (China) was analyzed by Mahmood et al. [19]. The authors put forward the hypothesis that there is an optimum seed rate to compensate the negative effects of decreasing N for balanced high yields and an improved NUE in wheat. The results revealed that the net photosynthetic rate, the stomatal conductance, the chlorophyll content, and the activities of metabolic enzymes significantly increased with increasing N levels and a decreasing seeding rate. The plant tillers, grain yield, dry matter before anthesis and N translocation, N agronomic efficiency (NAE), N recovery efficiency (NRE), and N uptake efficiency (NUPE) were highest in a combined treatment of N235 and SR180. However, N levels beyond 235 kg ha^{-1} significantly decreased the NAE, NRE, and NUPE. The authors concluded that 1 kg N ha^{-1} might be replaced by an increase of approximately 0.6 kg ha^{-1} SR. In addition, by using a combination of N and SR (N235 + SR180), it is possible to obtain the maximum yield of winter wheat and improve the NUE parameters [19].

The objective of another paper was to determine the best pruning level and N dose based on the agro-physiological characteristics of kaffir lime under mild shading [20]. The research was based on the need to fill the information gap regarding growth and yield under mild-shading conditions and specific N recommendations for leaf-orientated production of kaffir lime. The experiment was carried out at the Pasir Kuda experimental field of IPB University, Bogor, Indonesia. The plant materials were nine-month-old seedlings obtained using a grafting technique that combined kaffir lime (*Citrus hystrix* DC) scions onto rangpur lime (*C. limonia* Osbeck) rootstock. Four levels of N dosage were tested. The optimum N rate was determined based on a regression curve. A N-sufficient condition was achieved as the effect of 20 and 40 g N plant^{-1} application, producing a great growth and yield performance due to a high carbon assimilation rate. However, that does not automatically mean that a dose of 40 g N plant^{-1} is the best fertilizer recommendation, as 20 g N plant^{-1} is more efficient, with a relatively similar effect for increasing kaffir lime leaf production. With respect to pruning, a higher yield was obtained via leaving 30 cm of main stem above the ground, rather than shorter plants with a 10 cm main stem [20].

2.3. Balanced Fertilization as Key to Efficient N Use

Efficient N uptake, transport, and conversion into a crop depends on a good supply of plants with the remaining macro- and micronutrients [7]. The first publication dedicated to balanced fertilization described their results regarding fertilization in a rice–rice cropping system [21]. As rice is a nutrient-exhausting crop, its properly balanced fertilization is important to maintain a high productivity. The two-year experiment in a sub-tropical climate under the red and lateritic belt of the western part of West Bengal, India, was set up in a randomized complete block design with twelve treatments and three replications, with different rates of N:P:K:Zn:S application in both of the growing seasons, namely, Kharif and Boro. The results clearly indicated that imbalanced or insufficient nutrient application affects crop nutrient removal, thus affecting the growth and development of the plant. In addition, inappropriate nutrient supply over a long period reduces soil fertility, especially when a nutrient-exhausting cropping system, such as a rice–rice cropping system, is chosen. In this study, the recommended dose of nutrients was 80:40:40:25:20 and 120:60:60:25:20 kg ha^{-1} of $N:P_2O_5:K_2O:Zn:S$ in the Kharif and Boro season, respectively. To summarize, balanced nutrient management in cropping systems is a cost-effective and environmentally friendly approach to targeting agricultural sustainability [21].

In another paper, the authors focused on interactions between differentiated fertilization management and environmental factors and their influence on potato yields and selected soil parameters [22]. The fertilization treatments represent different management practices and include: (1) an unfertilized control, (2) the application of cow manure (FYM), (3, 4) a combination of manure and two different mineral nitrogen rates (FYM + N1, FYM + N2), and (5, 6, and 7) a combination of FYM and mineral NPK fertilizers (FYM + N1PK, FYM + N2PK, FYM + N3PK), which represents the combination of manure and all three major mineral fertilizers (against FYM + N treatments). The experiment was carried out on three sites (different soils) and during four growing seasons. Both the growing season and fertilization significantly affected potato yields at all locations. The authors proved that FYM application was always associated with higher yields. However, FYM application did not provide enough nutrients (N) to fulfil the yield potential of potatoes. Therefore, the addition of mineral N significantly increased potato yields, especially at less-fertile sites. The FYM + NPK combinations significantly improved yields compared to the FYM + N treatments. Thus, the obtained results clearly confirm the important role of P and K fertilization in increasing N productivity via both natural and mineral fertilizers.

The role of balanced fertilization in yield formation was also analyzed via two long-term experiments. The first was set up in 1954 in Prague and analyzed the effect of weather and seven fertilization treatments (mineral and manure treatments) on winter wheat grain yield and stability [23]. Winter wheat is one of the most important crops in the world. Hence, analysis of the response of wheat varieties to perennial fertilization is particularly important for food security. The authors analyzed 23 growing seasons. They showed that the grain yield was positively associated with the April precipitation, the mean daily temperature in October, and the daily maximum temperature in February. The yields were most stable between years when two fertilizer treatments were used that supplied a mean of 47 kg N ha^{-1} yr^{-1}, 54 kg P ha^{-1} yr^{-1}, and 108 kg K ha^{-1} yr^{-1}. The rate of N at which the grain yield was optimized was determined according to the linear-plateau (LP) and quadratic response models as 44 kg N ha^{-1} yr^{-1} for the long-strawed varieties and 87 kg N ha^{-1} yr^{-1} for the short-strawed varieties.

Another article included in this subsection presents the impact of well-balanced fertilization on the effective N fertilization of corn [14]. The objective of the study was the influence of the band application of a di-ammonium phosphate and ammonium sulfate mixture (NPS) on the possibility of lowering the total N dose. In order to assess the impact of fertilizing agents, seven nutrient efficiency indices and eight dry matter and N management indices were used. The total N uptake and NUE indices increased after band application. In addition, a trend of improved N remobilization efficiency and the N contribution of remobilized N to grain as a result of the band application of NP(S) was observed. The most effective use of N by corn was ensured via the use of an NPS mixture during the sowing of corn seeds (band application). From the point of view of the NUE indices, the optimal dose of N was 60 kg ha^{-1}. With broadcast fertilization and/or a further increase in the N dose, without the simultaneous use of P and S, the values of the NUE indices deteriorated, especially in the year with the highest content of N_{min} in the soil. Thus, a positive effect of the interaction of N and P(S) was confirmed in the conditions of soil rich in plant-available P.

Another publication concentrated on the improvement of N use by potato plants through the additional application of elemental sulfur, S^0 [24]. Potatoes require a good supply of S^0 for effective growth. Earlier studies showed that, in conditions of good S supply, a simultaneous increase in the NUE was noticed [25]. In this study, two main goals were set: (i) quantify the seasonal growth trends in the biomass of potato organs competing with tubers and (ii) evaluate the impact of S^0 on the in-season relationships within the biomass of potato organs. The research factors were two doses of N (60 and 120 kg ha^{-1}), elemental sulfur fertilization (control and 50 kg ha^{-1}), and different plant sampling dates (10-day intervals). It was found that the potato growth pattern coded at the onset of tuberization was a decisive factor for the dry matter partitioning between the potato organs during the subsequent tuber growth phase. The tuber yield-forming effect of added sulfur results from a balanced growth in stems during the ascending and the descending phase. At harvest, the average biomass of potato tubers on the main plot fertilized only with N was lower by 21% than that on the one receiving sulfur at the rate of 50 kg ha^{-1}.

In a methodological publication by Hu et al. [26], a hypothesis was formulated that the optimal fertilizer doses can be determined via yield–fertilizer rate response modeling. For this purpose, the authors analyzed dozens of experiments with peanut plants located on the North China Plain. Two fertilization treatments, namely, that used by farmers (FP) and optimized fertilization (OPT), allowed for the regional mean optimal rate (ROMR) method to be applied. The authors determined the optimum fertilizer rate using the 2^{o} regression curve. In order to assess the fertilization effectiveness, the authors used a number of indices: the RIEN (N reciprocal internal efficiency), PFPN (N fertilizer partial factor productivity), NUpE (N uptake efficiency), and NUtE (N utilization efficiency). The results of the experiments supported the hypothesis that the FP treatment with the OPT treatment, based on the RMOR method, promoted N use efficiency (PFPN and NUPE) and decreased the nutrient inputs from chemical fertilizer, especially N and P fertilizers, without the loss of peanut yield and NPK uptake. The research clearly shows that the RMOR method can be adopted in many countries and regions with widespread smallholder farms.

2.4. FUE and Foliar Fertilization

One way to provide plants with nutrients during the vegetative phase is foliar fertilization. This treatment allows for interventional (when deficiency symptoms appear) or preventive fertilization, taking into account the growth phases in which plants show the greatest sensitivity to nutrient deficiency. The method bypasses the stage of the transformation of nutrients in the soil, and thus reduces the potential regression of components and/or dispersion into the environment. In addition, through the use of small doses, a high fertilizer productivity is achieved [27]. There are insufficient data in the literature on foliar fertilization with phosphorus and, in particular, on plants of the *Fabaceae* family. The results published in this Special Issue broaden our knowledge on foliar P application and its influence on selected growth parameters, the production, and the quality of peas [28]. The effect of foliar P application on the photosynthetic parameters, seed yields, and quality of four pea genotypes (two normal-phytate cultivars and two low-phytate) was investigated in a pot experiment in controlled conditions. The effect of the pea lines on the foliar P fertilization was different. In the case of the normal-phytate cultivars, the seed production was enhanced via gradual doses of the P-fertilizer, except for the highest dose of phosphorus (P3). Low-phytate cultivars showed a positive reaction to the P3 dose. The authors concluded that foliar P application could be an effective way to enhance the pea growth in the P-deficient condition, with a direct effect on the seed yield and quality.

The research objective of another publication was to verify the effect of the foliar application of waste elemental sulfur (S^0) from biogas production in combination with conventional liquid UAN fertilizers applied in different ratios [29]. The reaction in maize was studied via a pot experiment. The following fertilization treatments were studied: control, UAN, UANS1 (N:S ratio, 2:1), UANS2 (1:1), and UANS3 (1:2). It showed that the application of UAN increased the N content in the plant and significantly affected the chlorophyll content (the N-tester value). The application of UANS had a lower impact on the N content and uptake than the application of UAN; however, it had a significant effect on the quantum yield of PSII. The authors conclude that the foliar application of UAN fertilizer in combination with S^0 in a 1:1 ratio seems to be a sensible way to optimize the nutritional status of maize, both in terms of the economics of biogas purification, where the waste sulfur is reused as a fertilizer, and for environmental reasons.

Apart from P and S, the most important component for N uptake and metabolism in plants is Mg. In agricultural practice, farmers use two basic Mg fertilization systems: (i) the in-soil application of Mg fertilizer using lime for acidic soils and magnesium sulfate for soils with an optimum pH; and (ii) foliar fertilization. In studies carried out by Potarzycki et al. [30], a hypothesis was formulated that winter wheat fertilized with Mg increases nitrogen fertilizer (N_f) efficiency, regardless of the method of application. In order to achieve this, the authors set a two-factorial experiment with three doses of Kieserite (0, 25, and 50 kg ha^{-1} of Mg) and two stages of foliar fertilization at the rate 2.4 kg Mg ha^{-1} (control; I; II; I + II). A full dose of nitrogen was 190 kg ha^{-1}. Twelve different parameters and indices (the total N accumulation, harvest index, partial factor productivity, nitrogen physiological efficiency, and others) were used to assess the impact of factors on the nitrogen efficiency (NUE) in wheat cultivation. The same set of indicators was used to assess the effectiveness of Mg fertilization. According to the study, the wheat yield increased as a result of the use of Mg. The method of application was of secondary importance. The yield gain, as a result of foliar fertilization with Mg fertilization, ranged from 0.6 to 0.9 t ha^{-1}, while, in the soil, its application resulted in a yield gain in the range of 0.4–0.7 t ha^{-1}. The main action of Mg, regardless of its application method, was the improvement of the index values characteristic for the NUE. The yield-forming effect of the applied Mg on the winter wheat was revealed via the increased N transfer to the grain.

In another publication included in this Special Issue, the authors investigated the effect of three foliar fertilizers (F, B, and C) and the mixture of the three (F + B + C) on the flower quality and the amount of new daughter corms produced by the five Gladiolus varieties in the climate conditions of the Carpathian Basin [31]. The *Gladiolus* genus is a perennial, monocotyledonous, geophyte, semi-rustic ornamental plant and includes about 260 species [32]. These plants are valued for the variety of shapes and colors of their flowers. However, they require appropriate growing conditions and the correct selection of varieties, in particular for degraded and saline soils. In the study, the authors used multicomponent foliar fertilizers that differed not only in the set of elements and their content but also in the presence of phytohormones. It should also be mentioned that N was included in each fertilizer. During the season, a total of four sprayings were carried out during plant development phases. The results of this experiment show that proper foliar fertilization can support and influence the growth, vase durability, and daughter corm production of some Gladiolus varieties. The highest yield of daughter corm production was observed with the mixture of the three foliar fertilizations (F + B + C). The result confirms that N productivity is stimulated not only via the dose of N but also via the appropriate balance of all nutrients [31].

2.5. FUE and Innovations on the Fertilizer Market

For many years, mineral fertilizers have been used to (i) ensure a good supply of nitrogen to plants, especially in critical phases; (ii) reduce the number of applications; (iii) reduce the nitrate content in plants; and (iv) limit nitrogen loss and reduce its negative impact on the environment [12]. In general, these fertilizers can be divided into two groups: slow-release fertilizers (SRFs) and controlled-release fertilizers (CRFs). With regards to N fertilizers from the CRF group, the effect of delaying N release is achieved through covering the granules with a different type of protective layer. Škarpa et al. [33] assessed the possibility of improving the efficiency of N_f and reducing its negative impact on the environment (N leaching) through the use of two CRF fertilizers: calcium ammonium nitrate (CAN) fertilizer coated with modified conventional polyurethane and CAN coated with vegetable oils. The influence of the CRF fertilizer was compared to that of the classic CAN. Three types of treatment were tested for both coated fertilizers: divided application (CAN, coated CAN), a single application of coated CAN, and a single application of CAN with coated CAN (1:2). The test plant was winter oilseed rape. The obtained results confirm that the application of coated CAN fertilizers increases the yield to a large extent, improves the efficiency of N fertilization, and reduces N losses, compared to the use of conventional CAN. In this study, a suitable method appears to be the application of a mixture of conventional CAN and coated CAN in a ratio of 1:2 during spring fertilization, ensuring a sufficient amount of rapidly releasing N during the regeneration of rapeseed and its slower release during further developmental stages. In terms of fertilizer production, oil-based polymer coatings on CAN fertilizer can be considered as an adequate replacement for partially modified conventional polyurethane [33].

The second publication on CRF fertilizers in this collection studied the possibility of enhancing the NUE in coffee cultivation (*Coffea arabica* L.). Freitas et al. [34] formulated a hypothesis that enhanced-efficiency N fertilizers and other fertilizers, such as ammonium nitrate and sulfate and prilled urea diluted in water, are options more suitable than conventional urea for reducing NH_3-N losses in coffee production systems. In order to validate the hypothesis, field experiments were carried out, in which the authors tested the following fertilization treatments: prilled urea, prilled urea dissolved in water, ammonium sulfate (AS), ammonium nitrate (AN), urea + Cu + B, urea + adhesive + $CaCO_3$, and urea + NBPT (all with three split applications), as well as blended N fertilizer, urea + elastic resin, urea-formaldehyde, and urea + polyurethane (all applied only once). The experiment with fertilizer treatments was conducted in coffee plantations in field conditions for two crop seasons in the Minas Gerais region, in Brazil. The treatments used in this study were applied at the 300 kg N ha^{-1} dose per year. The authors proved a significant influence of various fertilization combinations on urea losses. Except for urea + adhesive + $CaCO_3$ (27.9% of NH_3-N losses), all N-fertilizer technologies reduced NH_3-N losses compared to prilled urea. The lowest losses were observed for AS (0.6%) and AN (0.5%). The authors point out, however, that when choosing the right fertilization strategy (choice of treatment), the costs of the fertilizer application must be considered.

The problem of reducing losses of NH_3-N from fertilizers was also studied by Cassim et al. [35]. The authors assessed different nitrogen (N) fertilizer technologies in corn production systems through the characterization of N sources, NH_3-N volatilization losses, and their effects on the nutrient concentration and yield of corn grown in clayey and sandy soils in south Brazil. The following treatments were tested: control, three conventional N sources (urea, ammonium sulfate, and ammonium nitrate + calcium sulfate), and three efficiency-enhanced fertilizers (urea treated with NBPT + Duromide, urea formaldehyde, and polymer-coated urea + urea treated with NBPT and nitrification inhibitor, NI). The article features the physical properties of fertilizers obtained using scanning electron microscopy and X-ray diffraction. In general, the effect of N fertilizer technologies on N losses via the volatilization of NH_3-N was ordered as follows: urea > URP + Ur-NBPT + IN > Ur-NBPT + Duromide > Ur-formaldehyde > ammonium nitrate + calcium sulfate > ammonium sulfate. The studies confirmed that ammonium sulfate and ammonium nitrate have the least impact on NH_3-N losses (84 and 80% in relation to urea). Additionally, both fertilizers increased the corn grain yield. The yield increase in the clayey soil did not occur solely due to the reduction in losses via NH_3-N volatilization. Other factors, such as S and B supplementation and N release at a controlled rate to synchronize with the crop demand, also influenced the increase in corn yield. The authors also presented interesting data regarding the effect of fertilizer treatments on the macro-i micronutrient content and the chlorophyll concentration (SPAD) at the R1 phenological stage (silking). The results suggest that the use of nitrification inhibitors in soil, which leads to an increased concentration of NH_4^+, primarily reduces the uptake of Ca^{2+}, and then Mg^{2+}, to a lesser extent.

Biochars constitute a relatively new fertilizer on the market. In general, biochars are solid materials, rich in carbon, obtained from the thermochemical decomposition of organic biomasses. They may be treated as mineral fertilizers or as a component for the production of CRF fertilizers [12]. The in-soil application of biochars has a positive impact on carbon sequestration in soil and on reducing greenhouse gas emissions [36]. In addition, biochar application improves soil fertility and crop productivity. However, the literature does not provide sufficient data on the effect of biochars on the physiology of tomato yields. This gap is filled by the publication by Liu et al. [37]. The authors assumed that the improved agro-chemical properties of the soil using biochar and vermicompost had a positive effect on plant growth, selected physiological parameters, and the tomato yield. In order to verify this hypothesis, the authors set up an experiment, which scrutinized the effect of biochar (CK0%; BA3, 3%; BA5, 5%; by mass of soil) and vermicompost (VA3, 3%; VA5, 5%) on photosynthesis, chlorophyll fluorescence, and tomato yield under greenhouse condition. A number of parameters specific to photosynthesis and chlorophyll fluorescence in tomato plants were analysed. The optimal parameter values were obtained in the treatment with the highest rate of vermicompost (VA5). The treatments with BA registered lower values, but these were higher, however, than those with CK. In summary, the authors highlight that for one season of tomato production, the application of 3% vermicompost is considered economical with regard to improving photosynthesis, enhancing the WUE, and increasing the tomato yield.

2.6. Phosphor and Potassium Use Efficiency

Besides N, phosphorus is the second most important nutrient in agriculture. The need to improve the use of P from fertilizers by crops stems from two basic factors: (i) the limited resources of raw materials economically viable for exploitation; (ii) the adverse effects of the component's dispersion into the environment [38]. Opportunities to improve the use of P from fertilizers can be explored in various ways. It is important to create not only the optimal conditions for the mobility of $H_2PO_4^-$ ions (e.g., the soil pH) but also the right choice of doses and type of fertilizer. As the research of Santos et al. indicates [39], in order to improve the efficiency of P use, it is crucial to select the right variety to suit the environment/location. The authors investigated the additive and non-additive effects of commercially relevant traits for the popcorn crop (grain yield—GY, popping expansion—PE, and expanded popcorn volume per hectare—PV) in different conditions of phosphorus (P) availability in two locations in Rio de Janeiro State, Brazil. Six S7 lines previously selected (three efficient and responsive; and three inefficient and non-responsive for P use) were used as testers in crosses with 15 progenies from the fifth cycle of intrapopulation recurrent selection of UENF-14. The 90-hybrid analysis allowed the authors to determine the combination with the highest impact of dominance genes on performance and responsiveness in the use of phosphorus for the GY, PE, and PV traits.

Chlorine is an essential micronutrient for plants. Its content in soils used for agriculture is usually at a much higher level than the nutritional needs of plants. One of the reasons for this condition is the widespread use of potassium in the form of chloride salt (KCl). Excessive Cl content in the soil can reduce the yield and quality of many crops and thus reduce the efficiency of K from fertilizers. The species sensitive to excess chlorine in the soil include coffee plants. High concentrations of Cl are related to an increase in plant water, which favors an undesirable fermentation of coffee fruits [40]. A way to bypass the problem would be to use K_2SO_4. However, this fertilizer increases the cost of fertilization. In the article, the authors proposed a partial replacement of KCl with K_2SO_4 [40]. To achieve this, the authors investigated the effect of blends of KCl and K_2SO_4 fertilizers at different proportions and their influence on the yield, nutritional state, and chemical composition and quality of the coffee beverage. The research clearly shows that the K content in the leaves was not influenced by the application of blends of K fertilizer while the Cl content increased linearly with the KCl applied. Fertilization with KCl reduces the cup quality and the activity of the polyphenol oxidase, probably due to the ion Cl. Taking into account the yield of coffee plants, the optimal ratio of KCl and K_2SO_4 was 1:3. However, the highest score in the cup quality test was observed with 100% K_2SO_4.

3. Conclusions

Improving the use of nutrients from fertilizers (the FUE) is one of the most important goals of modern agriculture in the context of the increasing demand for food and the growing pressure on the environment. This Special Issue presents a number of possibilities and strategies to improve the FUE. According to the presented publications, most of the research focuses on the possibility of improving the use of N by plants through balanced fertilization. Only in a state of equilibrium between the supplies of N and other nutrients to the plant during the growing season is it possible to effectively exploit the yield potential of a cultivated plant. The balanced fertilization of plants is, therefore, the key to sustainable agricultural production. Balanced fertilization should be supported by other activities aimed at improving the FUE, such as shaping the optimal conditions for nutrient uptake, including the effective use of P and K from fertilizers, foliar fertilization, or the application of innovative fertilizers with a controlled release rate of nutrients and/or nitrification inhibitors. At the same time, the development of new technologies and fertilization strategies should be accompanied by progress in plant breeding that better utilizes natural and anthropogenic sources of nutrients.

Conflicts of Interest: The author declares that there is no conflict of interest.

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Review

Soil Fertility Clock—Crop Rotation as a Paradigm in Nitrogen Fertilizer Productivity Control

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Abstract: The Soil Fertility Clock (SFC) concept is based on the assumption that the critical content (range) of essential nutrients in the soil is adapted to the requirements of the most sensitive plant in the cropping sequence (CS). This provides a key way to effectively control the productivity of fertilizer nitrogen (N_f). The production goals of a farm are set for the maximum crop yield, which is defined by the environmental conditions of the production process. This target can be achieved, provided that the efficiency of N_f approaches 1.0. Nitrogen (in fact, nitrate) is the determining yield-forming factor, but only when it is balanced with the supply of other nutrients (nitrogen-supporting nutrients; N-SNs). The condition for achieving this level of N_f efficiency is the effectiveness of other production factors, including N-SNs, which should be set at $\leq$1.0. A key source of N-SNs for a plant is the soil zone occupied by the roots. N-SNs should be applied in order to restore their content in the topsoil to the level required by the most sensitive crop in a given CS. Other plants in the CS provide the timeframe for active controlling the distance of the N-SNs from their critical range.

Keywords: nitrogen; nitrate-nitrogen; nitrogen use efficiency; nitrogen-supporting nutrients; phosphorus; potassium; maximum attainable yield; soil fertility management

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1. Introduction—The Battle for Yield

The required increase in total food production of around 70% in 2050, compared to 2010, will largely depend on increases in the yields of crop plants, which serve as staple food for humans [1,2]. At present, meeting the demand for food in the next 28 years, in connection with the required reduction in greenhouse gas emissions, poses a major challenge for global organizations (e.g., the United Nations), the Food and Agriculture Organization (FAO), and national governments [3,4]. Russia's war on Ukraine has clearly highlighted the importance of net food producers, such as Ukraine, in stabilizing the global food market. In the 2020/21 season, this country exported 69.8 million tons of all cereals, accounting for 11.8% of the global export. Ukraine's share in global exports of sunflower seeds and oil exceeded 50% (52%). The war collapsed exports from Ukraine, not only of cereals and sunflower products, but also soybeans and rapeseed [5,6]. Strubenhoff [6] has indicated four groups of activities that can be urgently taken by world leaders to save the world from hunger. Concerning the national level, he pointed to the need to change the food policies of the EU and the USA. In the case of the USA, the author suggested reducing the production of biofuels. In the case of the EU, the author indicated the need to move away from policies on reducing the use of mineral fertilizers. The general conclusion formulated by Strubenhoff [6] was as follows: "For the time being, we need more production, not less. Climate objectives are good to save the planet, but we also need to feed the people on the planet". This should be seen as a motto for political and environmental players who truly understand the functioning of Planet Earth in a holistic, not reductionist sense.

The increase in food production by 2050 will in fact be the result of two main drivers, including the increases in arable land area and yields of main staple crops. The first, the main factor in covering the food gap by 2050—is in fact a key constraint, as evidenced by the wide discourse pursued by decision makers in the area of food policy. This goal, in fact, is limited by a lack of fertile soils. The potential resources for expanding arable land area are predominantly in the tropics. However, these soils, despite high natural fertility, require large inputs of the means of production [7,8]. Moreover, the destruction of the rain forest is expected to completely disrupt the Earth's climate [9]. Thus, in order to cover the food gap by 2050, the main challenge facing the world is to increase the yields of crop plants in "old agricultural areas" [10]. The share of the second—but dominant—factor in covering the food gap by 2050 (crop yields), has been estimated at over 80% [11,12]. There are four main factors that are considered crucial in actions oriented toward yield increases. The first is breeding progress. Meeting the 2050 target requires an annual increase in the yield of major crops such as wheat, maize, rice, and soybean at the level of 2.4% annually. This target, as reported by Ray et al. [13], will not easily be met, as the current yield increases of these crops are much below this target, reaching in relative terms only 67% for wheat, 42% for maize, 38% for rice, and 55% for soybean. The second factor is inherent in the effective use of mineral fertilizers and other crop protection measures. The actions taken by farmers should, however, be in line with the main assumptions of the concept known as Intensification of Sustainable Agriculture, which indicates the effective use of production means, including fertilizers [14]. The term "battle for yield," as proposed in the title of this chapter, in the current geopolitical context refers precisely to the productivity of the basic unit of plant production (i.e., a single field) which, in fact, defines the homogenous fertility unit of the field [15].

The third factor—and, indeed, a dominant factor in the food production sector—is the effective use of fertilizer nitrogen (N_f). The N_f consumption, as forecast for 2050, is 76% higher than in 2000 (181 vs. 103 mln t y^{-1}) [4]. In another study, the increase in the demand for N_f in 2050, compared to 2005, will be in the range of 43–73% [16]. The crucial problem with N_f use by farmers—both for production and, consequently, for the environment—is its low efficiency (recovery). Limiting the effectiveness of N_f to the right N dose, fertilizer approach, and even the timing of its application is a dramatic simplification of a complex problem [17,18]. A reduction in N_f consumption, in light of the drastic increase in N fertilizer prices in 2022, seems more realistic at present [19]. However, the sudden drop in N_f consumption, as observed in 2022 in Europe, could disrupt the global food production chain. The maintenance of the level of N_f consumption is crucial for food production in the old agricultural areas in the world [20]; for example, a simulation regarding reduced N_f consumption in the U.S. for maize and rice indicates a possible decrease in yields of 41% and 27%, respectively [21]. These theoretical considerations from 10 years ago should be considered, in the face of the current fertilizer crisis [6,19].

The fourth factor, which is decisive for the efficient use of production measures in agriculture, is the knowledge and skills of the farmers and their advisers, which are necessary to exploit the yield potential of the currently grown varieties. The real challenge for the farmer in the effective use of N_f is the correct diagnosis of the plant demand for N in the most critical phase(s) of the yield formation. At this particular stage of crop plant growth, a synchronization between the plant's requirements for N with its supply from soil (soil N resources plus controlled N_f application) is crucial for the formation of yield components. The farmer must know and recognize both the phases in which the plant builds up the yield components and the phases in which they are reduced. The importance of this issue can be presented through the examples of three crops. The first example concerns cereals, which deliver about 60% of carbohydrates and 50% of proteins to the world food market [22]. The key yield component determining the grain yield is the number of grains per unit area (grain density; GD). The most critical period of GD formation extends from the heading phase through flowering to the early milk (BBCH 71) [23,24]. Therefore, it can be called "the critical cereals' window". The second primary component of the yield is the grain weight (1000 grain weight; TGW), which is established during the grain filling period (GFP). This period extends from the early milk stage (BBCH 72) to plant maturity

(BBCH 90) [25]. Its impact on grain yield is much lower than that of GD [26]. For the second example, maize is a crop producing the greatest amount of food for humans or fodder for livestock [27]. The critical period of the primary yield's component formation is the stage of fifth leaf, in which the cob initials are formed. The key nutrient responsible for this process is the supply of N [28,29]. The third example is potatoes, the importance of which as food for humans has been growing rapidly [30]. The critical period for tuber yield establishment is tuberization [31]. The tuber yield depends on the number and weight of young tubers. These processes are driven by the supply of N, but also require a good supply of potassium (K) and phosphorus (P), at least [32,33]. These three examples allow us to conclude that the farmer's knowledge about the functions of N in plants is the absolute basis for determining an effective technological solution for the cultivated crops.

The basic questions to be asked here are as follows:

(1) Is the increase in nitrogen use efficiency (NUE) the real challenge for the increase in yields?
(2) How is the effect of nitrogen-supporting nutrients (N–SNs) on efficiency of N_f manifested?
(3) What is the required level of N–SNs in the soil, in order to maximize the N_f yield-forming effect?

The third question is essentially sets the goal for this conceptual article.

2. A New Paradigm of Nitrogen Use Efficiency Control—The Basis of the Concept

There are five general assumptions to consider before any discussion or forecast of crop production outcomes (i.e., the yields) by scientists, farmers' advisors, and food policy makers. First, the amount of solar energy reaching any part of the Earth's land surface is determined by its geographical location [34]. In agriculture, the basic unit of analysis is a single field or its homogenous production part, which is directly managed by the farmer [15,35]. Second, the accumulation of dry matter by a plant during its life cycle, its growth rate, dry matter partitioning between plant organs, and subsequent remobilization depend on the water and N supply. Third, the amount of available N in the plant rooting zone during the growing season is critical for the formation of yield components and, consequently, for the yield. Fourth, the uptake and use of N by the plant depends on the availability of other essential nutrients present in the plant's rooting zone. Fifth, the capacity of the soil and its potential to provide these nutrients to plants is limited. Their content in the plant natural growth milieu (that is, soil) is not infinite and, therefore, needs to be both controlled and supplemented by the farmer.

The effects of climatic and soil factors (environmental conditions) on crop growth and yield are manifested in terms of the maximum attainable yield (Y_{attmax}), which can be reached in well-defined geographic locations [36,37]. It is necessary to take into account that the use and impact of non-nutritional production factors on the grown crop is the result of a farmer's decision and/or legal limitations. Thus, on a specific field on a farm, the main issue for the farmer is to consider the effective management of N, which, in fact, depends in the current state of soil fertility. Sustainable management of N in the field should, therefore, be considered as a balance between necessary and sufficient conditions:

(1) The necessary condition is the actual yield, which is a function of the amount of available N in the rooting zone of the currently grown crop.
(2) The sufficient condition is the yield-forming functions of nutrients other than N to support the action of N by increasing its uptake and its use by the currently grown plant.

In fact, the necessary condition concerns the control of efficiency, which is the productivity of available N present in the soil zone occupied by plant roots during the growing season. The more detailed assumptions are as follows:

(1) A crop plant in a well-defined geographic area, provided stable environmental and nutritional conditions, can reach Y_{attmax}.

(2) The key production factor is N, present in the soil or/and supplied to the plant as fertilizer (natural, manure; mineral, N_f).
(3) All other nutrients, called nitrogen supporting nutrients (N-SNs), affect the Y_{attmax}, in relation to their relative deficiency in available form in the plant rooting zone.

These relationships can be expressed as a set of general formulae:

1. Actual yield:

$$Y_a = Y_{attmax} \times EN \quad (1)$$

2. Nitrogen Efficiency (EN):

$$EN = EP \times EK \times EMg \times ES \times \ldots\ldots \times ETi \quad (2)$$

where Y_a denotes the actual yield; Y_{attmax} denotes the maximum attainable yield; EN is the fractional value of NUE; and EP, EK, . . . , ETi are the fractional efficiencies of various N-supporting nutrients (N-SNs).

When the fractional value of an N-SN's efficiency index approaches 1.0 ($\leq$1.0), it indicates a sufficient range of content of the N-SN in available form in the soil. However, any deviation from 1.0 indicates a disturbance in the supply of a given nutrient to the currently grown crop, thereby reducing the NUE. Summarizing the above assumptions, it should be clearly stated that the key challenge for the farmer is to achieve high productivity of N fertilizer (N_f), but without a reduction in yield. This goal is achievable, provided that the critical level (sufficiency range) of soil fertility is achieved for N–SNs. Only at the equilibrium state between the supplies of N and N–SNs to the plant during the growing season—in particular, in the critical phases of yield component formation—is it possible to effectively exploit the yield potential of the currently cultivated plant.

3. Nitrogen—A Unique and Critical Factor in Plant Production

There exists a general consensus that the choice of a cultivar with well-defined yield potential is the basis of crop cultivation. Exploitation of the crop potential, however, essentially depends on the supply of water and N. For these reasons, these production factors are defined as yield-limiting [38,39]. These factors cannot be, however, treated as substitutes [40]. Water is a growth factor that regulates the plant temperature and, as a consequence, its whole metabolism and growth [41]. It has been well-documented that the amount of water available to the plant during the growing season is the result of both the water retention capacity of the soil and current precipitation. These two factors determine the Y_{attmax} under well-defined climate and soil conditions [36]. Water acts as a natural carrier of nutrients, both in soils and in the plant [42].

The plant is an autotrophic organism which, in order to close its life cycle, must be supplied with adequate amounts of both water and nutrients at well-defined stages of growth [43]. Plant growth can be defined as a set of processes in which both the plant and the soil—as its natural growth medium—interact with each other throughout the growing season. The importance of N for plant growth and yield results from its presence in key biological molecules [42]. The key N-dependent enzyme, which is decisive in the survival of life on Earth, is the ribulose bisphosphate carboxylase-oxygenase enzyme, simply called Rubisco (RuBP). Its key function is the capture and subsequent fixation of the CO_2 molecule, which is the basic substrate for the production of elementary sugar compounds [44,45]. The total mass of Rubisco in terrestrial plants has been estimated at $\approx$0.7 Gt. This enzyme constitutes 2.5–3% of the total leaf weight of leaves and about 50% of the total leaf proteins [46]. A hypothesis has recently emerged that Rubisco may also be treated a source of N during protein synthesis. This phenomenon is revealed only under conditions of excessive CO_2 capture by the plant in the circadian cycle [46].

N, mainly as nitrate ($N\text{-}NO_3$), also acts a local and systemic signaling molecule involved in the current regulation of the hormonal status and morphology of the plant [47,48]. For this reason, this inorganic N form has recently been termed a plant morphogen [49]. Clear evidence for the dominant role of $N\text{-}NO_3$ in yield formation is its influence on plant growth, which affects both the shoot and root system architecture [50]. The effect of N supply to the plant manifests itself in clear, visible changes in the architecture of the plant's canopy. As can be seen in Figure 1a, wheat plants grown on an N control plot (i.e., without N_f supply) presented stunted growth (i.e., dwarf stature), low weight and surface area of leaves, and pale green color. In contrast, plants well-fed with N were characterized by a well-developed shoot, high mass and surface area of leaves, and an intense green color. All of these plants, despite a significant difference in the architecture of shoots, were in the same phase of growth (i.e., booting; BBCH 40–49). This phase is the crucial for the development of yield structure and determines the number of fertile florets [51]. Excess N supply to the plant, as shown for maize, results in the establishment of more cobs per plant (Figure 1b). However, this does not mean a higher yield of grain. Excessive supply of N also results in excessive growth of non-productive plant parts, leading to a reduction in grain per unit area [52,53].

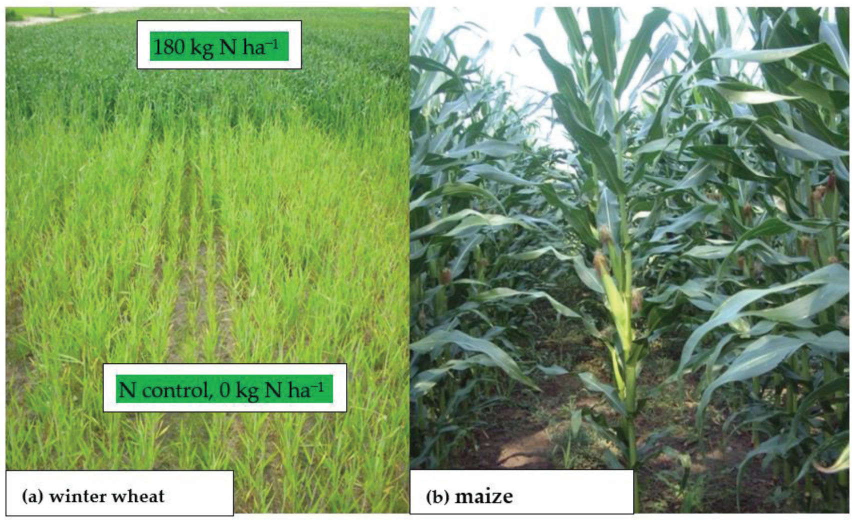

Figure 1. Impact of nitrogen fertilization on nutritional status of winter wheat and maize (photos by W. Grzebisz).

The introduction of new cereal phenotypes in the 1960s, such as varieties with reduced stem length, first for wheat and then for rice, significantly changed the shoot architecture (dwarfism of the shoot). These genetic modifications resulted in an increased harvest index (HI)—that is, the share of grain in the total shoot biomass—at the expense of the stem. Improvement of the harvest index (HI) is the greatest effect of the Green revolution, as it finally led to higher grain yields of cereals, including rice [54]. However, the exposition of dwarf genes has also caused a reduction in the root system size of wheat varieties, which is significantly smaller than that of the classic ones [55,56]. As a consequence, the semi-dwarf or dwarf growth mode of modern cereals varieties result in the higher yield, provided that the supply of nutrients (especially N_f) is high, and that the plants are strongly protected against pathogens [54,57]. It can, therefore, be concluded that the currently grown cereals, due to their high requirements for N on one hand, and their smaller root systems on the other hand, are extremely sensitive to the supply of nutrients responsible for the uptake of N from the soil. One of the proposed solutions aimed at the increase in nitrogen use efficiency (NUE) are new-generation varieties that are capable of developing deep root systems. The proposed ideotype of this root system—referred to as "steep, cheap, and deep"—assumes the effective uptake of water and dissolved nutrients (mainly nitrates) [58,59]. A reorientation of the current breeding approach is urgently required in intensive production systems, where high rates of N_f are typically applied. It can also provide a good solution in areas with frequent periods of drought, regarding the main phases of plant growth.

4. Nitrogen-Supporting Nutrients

Plant growth and productivity are the result of the action of about 20 elements that must be present in the soil to complete the plant's life cycle. The biophysical functions of plant-related elements have been well-documented and presented extensively in textbooks and review articles [60–62]. Not all of these elements are considered as nutrients, but all of them have a positive impact on the yield of crop plants [63]. A typical example is titanium (Ti), the positive effect of which on many crops has been recently documented [64].

N, considered especially in the form of nitrate (as discussed in the previous section), is the key nutrient, affecting both the rate of plant growth and the formation of yield components. The key evidence, in addition to that discussed above, is the response of the yield to the application of N–P–K fertilizers in various mutual fertilization systems. The effect of the interactions between N and other basic nutrients has been well-presented in long-term static fertilization experiments [65–67]. As shown in Figure 2, winter rye cultivated on Luvisol in a 7-course crop rotation (including two years of alfalfa) for 40 years yielded on plots without K (NP) or P (NK) only 6% and 5% less than on the NPK plot. The lack of both nutrients (i.e., K and P), as evidenced on a plot fertilized only with N, resulted in a yield drop of only 2.5%. The yield on the absolute control (AC) plot (i.e., the plot without application of any fertilizer, mineral or organic) for 40 years, was 30% lower than that on the NPK plot. Moreover, the same level of yield was recorded on plots fertilized only with P or K. Slightly greater differences between fertilization treatments were recorded for spring barley. Relative yield reductions were: −10%, −7%, and −42%, for NP, NK, and AC, respectively, as compared to NPK. The same yield level as for AC was noted on plots fertilized only with P or K. It must be added that the yields on the N plot were lower by 10%, compared to NPK [65]. The above-documented trends of plants grown on the naturally low fertility soil (Luvisol), with respect to different combinations of basic nutrients, have been supported by data from fertile soils, such as Entisol in the Netherlands (calcareous Entisol, containing 30% clay, 10% $CaCO_3$. The obtained data indicated that a lack of K fertilization over a period of 28 years did not adversely affect the yield of four crops grown in four-course crop rotation (sugar beets, spring barley, potatoes, and winter wheat) over 28 years. The lack of P was much more important, as its lack reduced the yield of sugar beets by 11%, but those of potatoes and spring barley by only 7% [68]. These two examples clearly show that the main source of P and K for crops is soil.

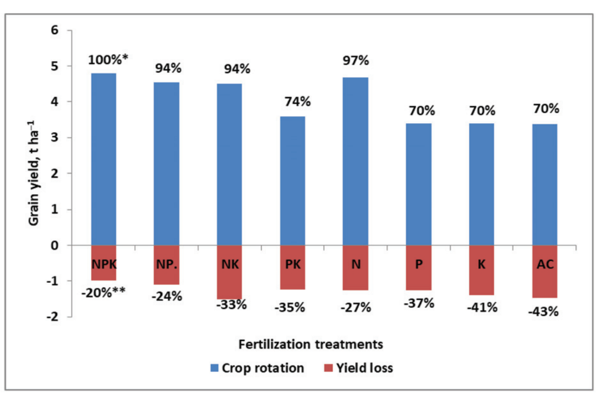

Figure 2. Effect of fertilization variants on yield of winter rye grown in 7-course crop rotation and monoculture (based on [65]). Legend: * Yield on the NPK variant = 100%; ** yield reduction—yield gap due to crop monoculture.

All plant nutrients support plant growth and yield formation through their impact on the productivity of N which, to a great extent, depends on the application of N_f Figure 1a,b. It can be concluded that it is unrealistic to expect an increase in the yield of a modern variety, as discussed above, without delivering N from external sources, whether natural (e.g., manure) or mineral. For these reasons, all nutrients affecting plant growth and yield can be called "nitrogen-supporting nutrients" (N-SNs). Based on agronomic practice, the whole set of N-SNs can be divided into four groups: (i) basic macronutrients, such as potassium (K) and phosphorus (P); (ii) secondary macronutrients, including magnesium (Mg), sulfur (S), and calcium (Ca); (iii) micronutrients, such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (N), molybdenum (Mo), and chloride (Cl); and (iv) the beneficial group, composed of nickel (Ni), sodium (Na), silicon (Si), and titanium (Ti) [61,69].

The second important stage in the development of an economically and environmentally sound fertilization system using N_f requires knowledge regarding the patterns of accumulation of N-SNs during the growing season. The classic pattern for high-yielding winter oilseed rape (WOSR) is presented in Figure 3. There are some characteristics "hot-points" in N-SN in-season patterns that require special attention by the farmer, based on the course of N accumulation. The most important points are:

(1) The higher accumulation of K over N during the whole season of WOSR growth:
   a. Starting with the rosette stage in Spring;
   b. Achieving the maximum K uptake over N at the full flowering stage (K_2O:N as 1:1.6);
   c. Declining from flowering to maturity (K_2O:N as 1:1).

(2) Slow early growth in P uptake, continued up to the inflorescence stage (BBCH 50), followed by rapid ingrowth, lasting until the full flowering stage (BBCH 65), and then smoothly decreasing up to maturity.

(3) A similar pattern for Mg as for P, but at a much lower level.

(4) A spectacular pattern of Ca accumulation. Its uptake increases sharply at inflorescence, reaching a maximum at the end of the pod growth stage.

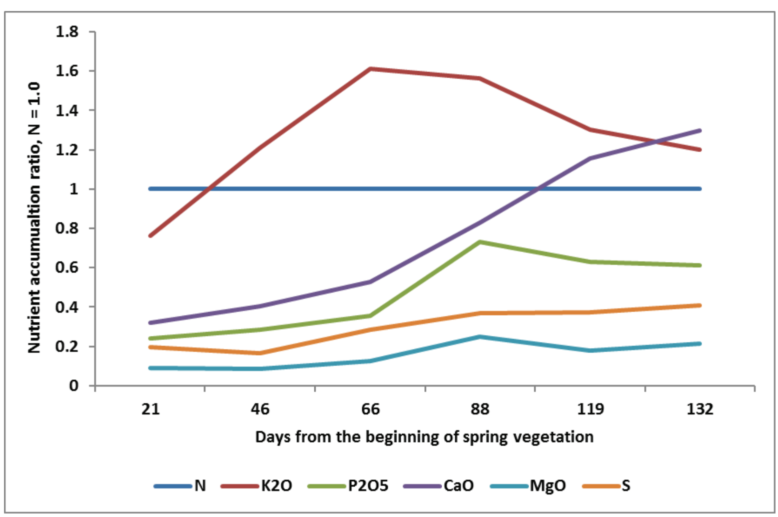

Figure 3. Patterns of nutrient accumulation during the growing season by high-yielding winter oilseed rape (simulation based on [70]).

The presented patterns of N, P, and K accumulation by WOSR during the growing season, obtained at the end of the 20th century, were very similar to their trends observed in the 1980s [71]. The same pattern has been observed for high-yielding rice [72]. The effective production of maize depends on the supply of K and N during the period preceding flowering, but requires stabilization of K accumulation up the milk stage [73]. It can be concluded that the high yield of the seed crops can be achieved, provided that the K:N ratio is higher than 1.0 during the vegetative phases for seed plant growth. In the light of the available data, the maximum K:N should be revealed for the high-yielding crop just before the end of the linear phase of its growth [74]. The seasonal pattern of basic nutrient accumulation by legumes is only slightly different. As observed for soybean, the maximum K was at the late vegetative stages, and the level then decreased smoothly, while the accumulation of N, P, Ca, Mg, and S progressed through to maturity. Moreover, the maximum K:N ratio was 0.6:1 [75].

5. Potassium

The biophysical functions of K are well recognized and described [76,77]. The most important functions of K in crop production are those that affect (i) nitrate-nitrogen uptake, (ii) protein synthesis, (iii) water management (i.e., control of the stomata circadian rhythm), (iv) growth of vegetative organs (i.e., transport of assimilates from leaves to the new buds and tissues), and (v) yield formation (i.e., transport of assimilates from leaves to growing fruits). All of these functions are inherent in the plant's growth cycle [72,78,79].

The deficiency of K during the growing period of the cultivated plant adversely affects N uptake and, thus, photosynthesis, leading to a reduction in assimilate production [80]. K deficiency in the first stages of plant growth reduces the growth of single cells. As a consequence, the growth rate of new tissues and organs is reduced (Figure 4a); this applies to both roots and shoots. The reduction in shoot biomass is slightly lower than that in root biomass [80,81]. As documented for sugar beet, the growth rate of fibrous roots is much faster in soil rich in available K, compared to the soil with medium content [82]. The rate of plant cell expansion depends on the action of auxins, the concentration of which in the plant and transfer to the roots is strongly related to the availability of N-NO_3 in the soil [49,83]. The stunted stature of crop plants is the most striking visual symptom of K deficiency (Figure 4). In maize, the length of internodes is drastically reduced during the phase of shoot intensive growth (Figure 4b). In cereals, the classical symptom is the same (i.e., reduced length of the stem). The secondary outcome, which is important in grain production, is the reduced density of ears (Figure 4a). This yield component will not be compensated for by a larger number of grains per ear or weight, thus directly leading to a significant decrease in grain density [24,84].

Crop plants differ in their demand for K. A proposed grouping of crop plants, according to K accumulated at harvest, and proposed recently by White et al. [85], has significant weaknesses. In general, it is true that cereals or legumes accumulate less K than dicotyledonous crops (leafy, root, or tuberous plants). It cannot, however, be concluded from this that cereals are tolerant of low soil K fertility level. It has been well-documented that cereals and cruciferous crops develop a large and intensive root system which, in turn, increases the absorption area of the plant for K uptake [86,87]. This specific plant feature determines the rate of K uptake under critical conditions, such as low content of available K or mild water stress. Sandy soils, as compared to loamy soils, as a rule are poorer in available K. Moreover, a decrease in the content of available water results in a much faster decrease in the coefficient of K effective diffusion [82,88,89]. The greater sensitivity of dicotyledonous plants to the level of soil K fertility is the result of two reasons [74,86]:

(1) Higher demand for K in the linear phase of growth;
(2) Much smaller root system, especially root length density.

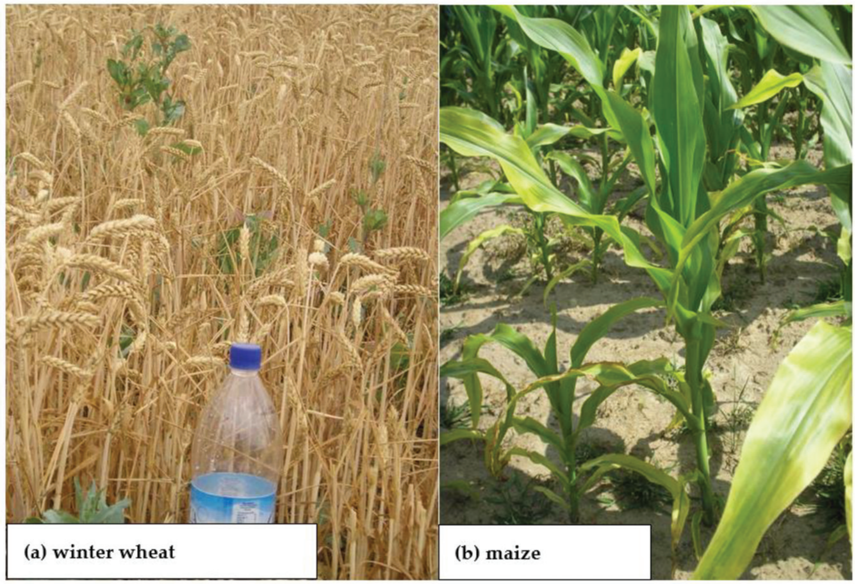

Figure 4. The stunted stature of plants due to potassium deficiency is the primary signal of yield depression in crop plants. (photos by W. Grzebisz).

Numerous scientific articles and even academic textbooks have presented—seemingly true—opinions regarding the quantitative dominance of N uptake over K accumulated by crops during the growing season [61,90]. This opinion can be applied to low-yielding crops, due to the deficiency of K in the linear phase of the plant growth [91]. In the case of high-yielding crops (i.e., those which are able to exploit the potential of cultivated varieties), the dominance of K uptake over N has been well-documented (Figure 3; [72,82]). The classic example is maize [53]. This crop takes more K than N during the vegetative growth. This opinion is also in accordance with a study on nutrient accumulation by wheat in India [92]. The authors clearly showed that the unit accumulation of K was higher than N, and the optimal N:P:K ratio in the plant dry matter for high-yielding wheat was as 6.6:1:8.1.

Based on the amounts of key nutrients accumulated by winter oilseed rape (WOSR), three regression models of the seed yield have been developed [93]. It is necessary to emphasize that the yield of WOSR ranged from 2.223 to 5.807 t ha^{-1}. The obtained regression models were as follows:

$$\mathrm{N} \rightarrow \mathrm{Y} = 0.12\mathrm{N} + 1.192 \qquad \text{for n = 18, } R^2 = 0.69 \text{ and } p \leq 0.01, \tag{3}$$

$$\mathrm{K} \rightarrow \mathrm{Y} = 0.007\mathrm{K} + 1.6 \qquad \text{for n = 18, } R^2 = 0.89 \text{ and } p \leq 0.01, \tag{4}$$

$$\mathrm{Mg} \rightarrow \mathrm{Y} = -0.005\ \mathrm{Mg}^2 + 0.39\ \mathrm{Mg} - 2.242 \qquad \text{for n = 18, } R^2 = 0.61 \text{ and } p \leq 0.01 \tag{5}$$

The final K uptake, regardless of the seed yield and weather conditions during the research, always exceeded that of N. The linear course for N and K clearly indicates that both nutrients were limiting factors for the yield. Moreover, the strength of the effect of N on WOSR yield, according to the R^2 coefficient, was much weaker compared to that of K. This model of N and K accumulation by seed crops is not new, having been defined about 40 years ago for winter wheat and WOSR [71,94].

The seemingly contradictory opinion regarding the impact of N and K on yield is explained in Figure 5. The amount of K in winter wheat (WW) during the Yield Formation Period (defined by the linear increase in dry matter)—a mega-phase covering the main phases such as stem elongation, booting, and heading—exceeded, regardless of the water conditions, the amount of N taken up. Moreover, the K:N ratio up to the beginning of wheat flowering for irrigated wheat was higher than 1.0. A sharp drop in K accumulation was revealed during the grain filling period. The course of N was significantly different from that of K, showing a net increase in its accumulation up to the early milk stage (BBCH 72), then decreasing slightly. These two (partly opposing) trends resulted in the K:N ratio narrowing at wheat maturity. The same trend of K accumulation has been recently observed in barley [95]. The presented data clearly explain the discrepancy in published data regarding the trend of K accumulation by high-yielding crops.

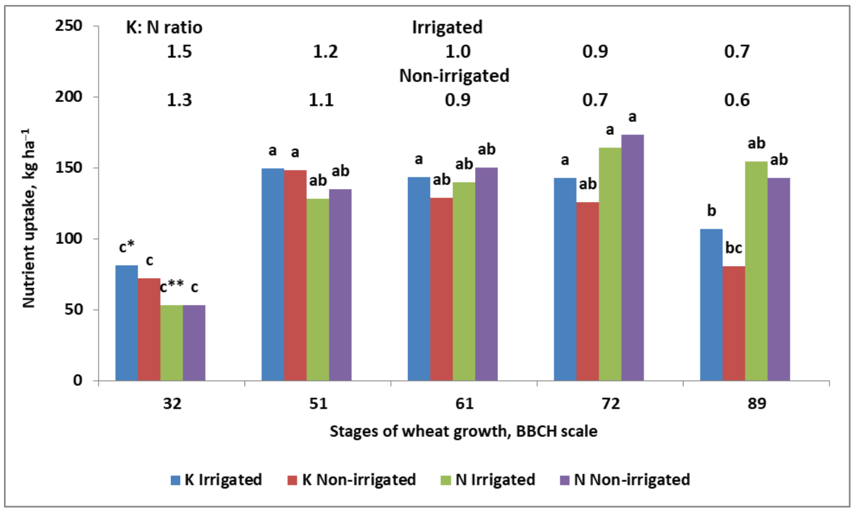

Figure 5. Nitrogen (N) and potassium (K) accumulation by winter wheat in critical stages of yield formation under various water conditions; means of three growing seasons (Grzebisz, not published). Legend: The different letters indicate significant differences between the treatments ($p \leq 0.05$); * irrigation and ** non-irrigation conditions of wheat cultivation, respectively; K, N, potassium and nitrogen, respectively.

The importance of K for yield formation is to be considered through the expression of yield components. It has been well-documented that K accumulation in seed crops reaches its maximum before flowering (Figures 3 and 5). In seed crops, this period is crucial for establishing the seed/grain density, which is treated as the key yield component [96]. It should be clearly stated that an adequate K supply to the seed crop during the Yield Formation Period (YFP) supporting N supply is the prerequisite of high yield. The presented opinion is neither a hypothesis nor an assumption, but a conclusion from our own studies and supported by available literature sources. The excessive uptake of K by wheat may result, however, in GD reduction, consequently leading to a decrease in grain yield [97]. This phenomenon has also been observed in maize and dicotyledonous plants, such as potato [52,98–100]. The unexpected effect of excessive K uptake can be explained by the accelerated supply of N to plants due to co-transport of NO_3^- and K^+ ions through the plasma membrane [62,80].

The role of K in the transportation of assimilates in the phloem has been well-documented [60]. The survival of seeds/grain in the period from setting up to the watery stage is inherently related to the supply of assimilates [101]; therefore, it can be concluded that K is responsible for the final seed/grain set. This concept is supported by the impact of the accumulated K on yield components, as shown in Equation (1). As recorded by Grzebisz at al. [93], K uptake at the beginning of WOSR flowering was the key factor, positively affecting the seed density (SD), while negatively affecting Thousand Seed Weight (TSW) at the same time:

$$SD = 203.3K + 3462 \text{ for } n = 18, R^2 = 0.92, p \leq 0.01 \quad (6)$$

$$TSW = -0.04K + 691 \text{ for } n = 18, R^2 = 0.48, p \leq 0.05 \quad (7)$$

The first equation fully corroborates the opinion of Pan et al. [102], who clearly stated that a deficiency of K reduces the sink size capacity. The second equation clearly confirms the phenomenon known as the dilution effect [103], concerning the dilution of nutrients in seeds, including N. Most important is the fact that the productivity of 1 kg of N_f increased from 14 to 24 kg seeds kg^{-1} N_f for K uptake of 178 and 504 kg ha^{-1} and seed yield of 3.0 and 5.14 t ha^{-1}, respectively [93]. Potatoes are considered to be a K-sensitive crop, in terms of both yield and tuber quality [104]. Studies on the effect of the N $\times$ K interaction on tuber yield have clearly shown that an increased dose of K fertilizer can significantly increase N productivity. This is an important premise for reducing the N_f dose [105,106].

6. Phosphorus

As in the case of K, the biophysical functions of P in plants are well-known and understood [60]. Three of its key functions can be treated as essential in agricultural practice. The first one concerns the biochemical energy (i.e., adenosine triphosphate; ATP), which is a high-P-energy compound. The synthesis of ATP is completely dependent on the supply of P to a plant. The generated energy is used in all plant energy transformation processes, starting with uptake of nutrients, and then their transport and transport of assimilates between the plant's organs. At the end of this (energy and matter) transformation chain, the accumulation of organic compounds takes place in the main crop products (e.g., seeds, grains, roots, tubers, fruits) [107,108]. The second key function of P is the synthesis of nucleic acids (DNA and RNA), constituting 40–60% of the organic P pool in the plant. These compounds are components of genes and chromosomes, constituents of the plant's genetic code. For this reason, they are responsible—among other aspects—for crop production; that is, the production of new generations of plants through seeds and grains [109]. The third crucial function of P, which is important in plant production, is phytin. This is a P storage compound in seeds/grains. The content of P in seeds is important, stimulating their germination and plant vitality in the early stages of growth [110,111].

Phosphorus is taken up by the plant from the soil solution as an orthophosphate ion ($H_2PO_4^-$, Pi) [112]. The amount of P needed to maintain the optimum rate of plant growth is much smaller, compared to N and K [85]. In general, the P requirements of non-seed plants is much lower that of the seed- or fruit-producing plants, ranging from 14 to 40 kg P ha^{-1} [107]. As with nitrates, the uptake of orthophosphate ions is an energy-dependent process. The key reason is the high P concentration gradient of 10,000 between its concentration in the plant cell (cytosol) and the soil solution [113]. The uptake of P from the soil is, first of all, a function of the plant root density (RLD; cm roots cm^{-3} soil). The content of available P in the soil solution is the second factor determining its acquisition from the soil [114,115]. The mechanisms of P extraction from the soil are diverse, depending on the current P nutritional status in the plant. Plants deficient in P trigger a number of processes, such as investment in the RLD and root surface area, symbiotic associations with arbuscular mycorrhizal fungi, rhizosphere acidification, and activation of phosphate transporters in the plasma membrane. All of these processes undergo acceleration when Pi concentration in the soil solution decreases [116–118].

Phosphorus deficiency results from low Pi supply to the plant root, due to its low content in the soil solution or unfavorable environmental conditions, including low temperature, water shortage/soil drought, and low soil pH, among others [119]. The visual symptoms of P deficiency—regardless of the plant species—can be seen on older leaves as bluish–violet discoloration, leading frequently to plant death due to disturbances to basic physiological processes (Figure 6a). These symptoms, appearing in the early stages of plant growth, are an indirect signal of a deep disturbance in N metabolism [120]. Crop responses to P deficiency manifest as growth inhibition, even leading to a failure in development of reproductive organs, which is often observed in maize (Figure 6b). A mild deficiency causes a temporary, short-term slowdown in plant growth [121]. The first plant response to a slight P deficiency is very specific, being manifested by the ingrowth of roots at the expense of the shoot biomass [108,122]. In a P_i-rich growth milieu, plants take up excess P to their needs, accumulating it an inorganic form. This pool is used during the growth of reproductive organs, such as seeds, grains, and tubers/roots [109,123].

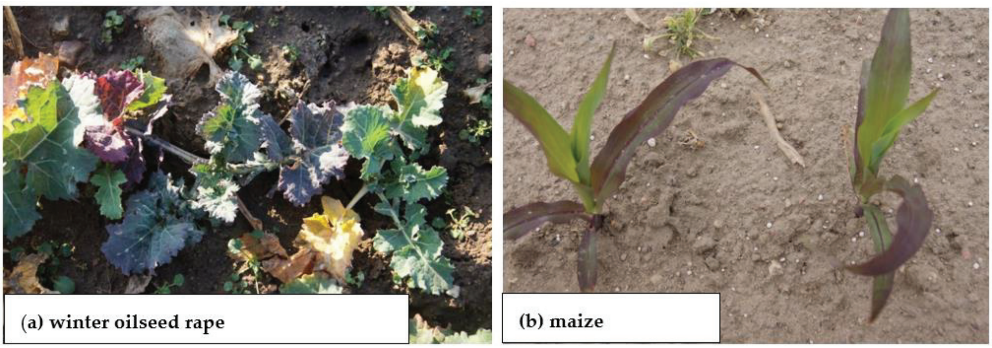

Figure 6. Symptoms of phosphorus deficiency in two different species grown in humid climate zone: (**a**) violet plants of winter oilseed rape are not able to conduct photosynthesis); and (**b**) violet maize plants at BBCH 33 often fail to develop a cob. (Photos by W. Grzebisz.)

Phosphorus yield-forming functions are best recognized for seed crops for which two critical stages have been identified. The first, minor function refers to all plants, and appears in the early stages of plant growth, being an important factor influencing the growth rate of roots and shoots [111,118]. For this reason, in plant production, a starting dose of P fertilizer is suggested to be applied, regardless of the content available P in the soil [124]. The second critical period for P uptake by dicotyledonous crops is poorly understood. In root and tuber crops, the increase in P accumulation is associated with the stage of intensive sugar or starch accumulation (Figure 7; [123,125,126]). As has been reported by Barłóg et al. [126], $\frac{1}{4}$ of the recommended P rate for sugar beets is sufficient to achieve a moderate yield ($\approx$60 t ha^{-1} FW); however, in order to exploit the sugar beet yield potential in Poland ($\approx$80 t ha^{-1} FW of storage roots)—which significantly depends on weather—the full P rate is needed. The key reason for this is that the storage yield depends on the supply of P to the plant during the late stages of growth. The advantage of a lower P dose (as shown in Figure 4) is the cessation of N accumulation in storage roots, resulting in both earlier sugar beet technological maturity and a lower content of so-called harmful N compounds [126].

In the case of seed plants, the main period of P requirement begins at the onset of flowering (Figure 3; [71,94]). Most of the P accumulated in the vegetative parts of these plants is then stored in seeds/grains. In a mature seed crop, between 85–95% of the total accumulated P is in seeds/grains [127,128]. The P remobilization efficiency ranges from 60–85% [129]. The degree of depletion of P in vegetative plant parts by the growing seeds/grain is not limited by its content in those organs, as has been suggested by Veneklass et al. [109]. In fact, it depends on the number of seeds/grains per plant that act as a physiological sink [127]. The key reason for the excess of P in vegetative plant parts is not the low rate of P remobilization from vegetative plant parts, as suggested by Wang et al., [130], but the low requirements of the growing seeds/grains [127].

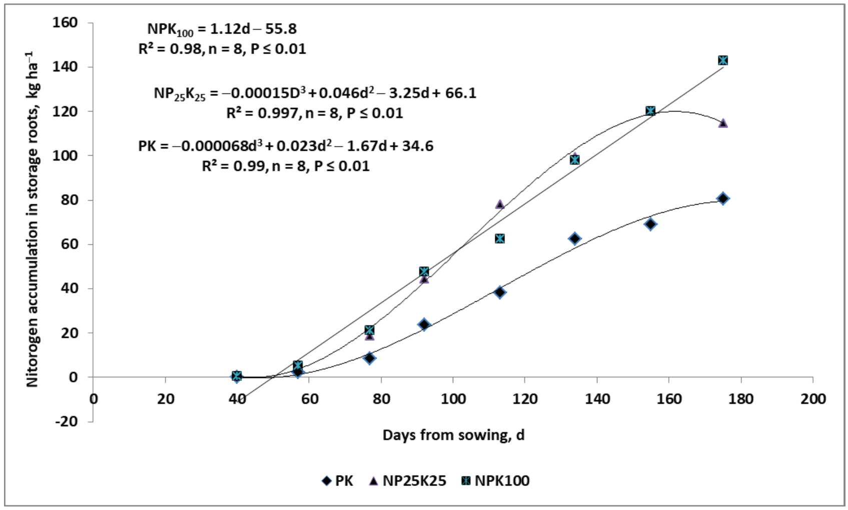

Figure 7. Effect of NPK fertilization systems on nitrogen accumulation in storage roots of sugar beets (based on Szczepaniak et al. [125]). Legend: PK—NPK100—the applied amount of P and K; N control; NP25K25—P and K applied at a dose of 25% compared to NPK100.

For low-yielding seed plants, and such a model dominates in the world, the P plant resources accumulated before flowering are sufficient to ensure even a moderate yield. The essence of the matter is the dilution effect that P is subject to in the reproductive organs of the plant [127,131,132]. However, a completely different model of the P management functions in high-yielding seed plants. In the first stage of reproductive organs growth, P is effectively re-mobilized and then re-translocated from the vegetative plant parts to the growing seeds/grains. The P resources accumulated in the plant before flowering, as a rule, are sufficient to cover the needs of the reproductive plant's organs. One of the reasons for this is the high P plasticity in seeds/grains, expressed by a very high rate of its dilution. This phenomenon has been observed for maize, wheat, rice, and winter oil seed rape [108,127]. In the second stage of seed/grain growth, the increased demand for P can be covered by P taken up by the plant from the soil [108,133]. This strategy is especially important for rice, which takes up 40–70% of the total P from the soil during the grain-filling period [129].

The reliability of long-term experiments for the assessment of P management by plants is limited by too high doses of P fertilizers used annually [65–67]. As a result, the recovery of P, even assessed over a long period, is typically low. As has been reported by Buczko et al. [66], the annual rate of P ranged from 10 to 210 (with an average of 60.7) kg ha^{-1} y^{-1}. The relative increase in yield, even on soil naturally poor in the available P, did not exceed 10%. Consequently, these data cannot be used to determine the sufficiency level of available P (P-SL) in the soil for the tested plants. A high P recovery (P-R) is usually expected in soils poor in available P. As shown in Figure 8, wheat responded to P fertilization up to 45 kg P_2O_5 ha^{-1}. This P dose increased the content of available P in the soil to a level sufficient for the maximum yield of wheat in this particular study. There was no increase in the yield above the range of 16–18 mg P kg^{-1} soil. Above this range, there was no response of wheat to an increase in the content of available P just before flowering [134]. In the presented example, the P recovery was 65% in the variant with 15 kg P_2O_5 ha^{-1}, 50% in the variant with 45 kg P_2O_5 ha^{-1}, and only 16% in the variant fertilized with 150 kg P_2O_5 ha^{-1}. Moreover, in P-R, starting from a plot of 45 kg P_2O_5 ha^{-1}, this perfectly fits into the power function:

$$P - R = 1751P_f^{-0.94} \text{ for } R^2 = 0.999 \text{ and } n = 8 \tag{8}$$

This example clearly demonstrates that P_f rates above that required for P–SL do not affect crop yield. Excessive P content in vegetative wheat parts, above P–SL, indicates insufficient size of wheat sink (the number of grain per unit area) to utilize these resources. This means that, for current wheat varieties, it is necessary to prepare new plant nutrition standards. Those that were developed 40–50 years ago are not reliable in current agricultural practice [135]. In the presented case, however, the most important fact is that the partial factor productivity of N_f (PFP-P_f) increased from 28 to 45 kg grain kg N_f^{-1} in the control P plot and 45 kg P_2O_5 ha^{-1}, thus determining the appropriate P-SL range.

Two conclusions summarize this section: first, the shortage of P significantly reduces, while its excess supply does not affect, N productivity; second, the P_f rate should adjusted to the soil level of availability that maximizes the productivity of N_f of the currently grown plant.

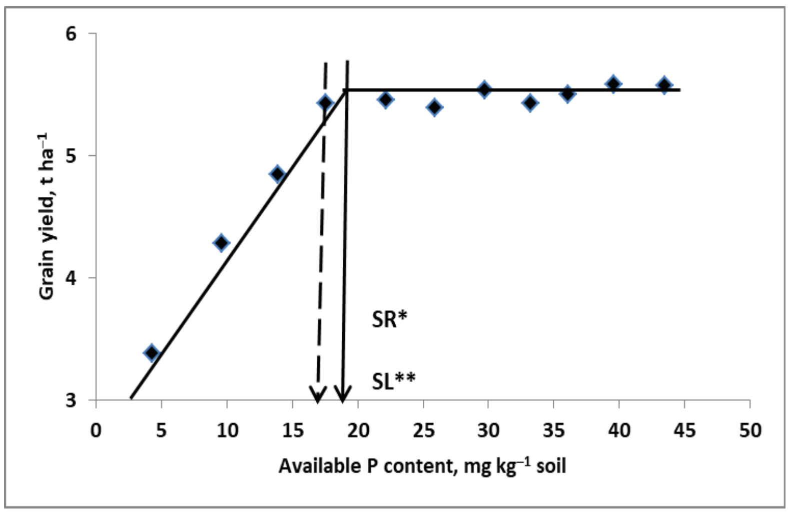

Figure 8. Yield of wheat response to the content of available phosphorus (based on [134]). Legend: * SR, ** SL, phosphorus sufficiency range, sufficiency level of the content of available P (determined by Olsen method).

7. Efficient Nitrogen Management—The Soil Fertility Clock Concept

7.1. Definition of the Concept

The role of N_f in crop production, which is the absolute basis of food production, results from its effects on the rate of plant growth, partitioning of assimilates between plant parts, formation of yield components, and quality of plant products [136]. The dominant impact of N on the life cycle of crop plants, as presented above, is justified at three levels of organization of the plant production process: (i) biochemical, (ii) physiological, and (iii) agronomic.

The key productive function of all other nutrients is to support the actions of N and, in fact, to control its productivity. The nitrogen use efficiency (NUE) is defined as the amount of main product per unit of N taken up by the plant during its growing season [137]. For this reason, any sophisticated attempt to calculate efficiency indices for K, P, or even for other nutrients is useless for a farmer. The calculation is useful, but only for understanding basic biophysical processes in crop plants [85,138,139]. The supply of N-SNs to the currently grown plant should be maintained at the level that maximizes—and not reduces—the yield-forming effects of N. It is necessary to take into account that the farmer cultivates plants in a specific sequence, called crop rotation. Therefore, the key goal of the farmer is to determine the critical level of soil fertility, not for all crops in rotation, but for the most sensitive plant to the nutrient, decisive for N productivity. This is the key challenge for the farmer, which should be treated as a necessary condition aimed at the development of an effective N_f management system for both farm economics and without imposing negative pressure on the health of the environment.

7.2. Maximum Attainable Yield—A Farm Production Goal

The potential yield of the grown plant—that is, the yield achieved under optimal environmental conditions (climate + soil) and rational management of the applied resources—is a theoretical term. This term, however, defines the production target, which may not be achievable in real production conditions [140,141]. Despite this, farmers need data on the maximum yields of crops that are grown in the geographical area of their production activity. This forms the basis for assessing the distance from the actual yield (Y_a) to the realistic production target, namely, the maximum attainable yield (Y_{attmax}). There are several ways to calculate or forecast Y_{attmax} and Y_a. There is no doubt that the most important factor determining N uptake from the soil solution is water, the carrier of ions arriving at the root surface [60]. Therefore, one of the most frequently used methods for Y_a determination relies on water productivity, in terms of water use efficiency (WUE). This method assumes a fixed amount of yield per unit of water [142]:

$$\mathrm{WUE} = \frac{Y_a}{ET_a} \tag{9}$$

where Y_a is the actual yield (kg, t ha^{-1}) and ET_a is the water use (i.e., water transpired by a plant or evaporated from the bare soil; mm, m^3);

This method assumes that a plant's yield increases with an increasing amount of available water. Hence, the yield defined in this way is called "the water-limited yield" (WLY) [142]. This method has been modified by Grzebisz et al. [82], as follows:

$$\mathrm{WLY} = \mathrm{TE}\,(\mathrm{R} - \Sigma \mathrm{E_s}) + \mathrm{WR} \tag{10}$$

where TE is the transpiration efficiency (TE = k/VPD; k is the biomass transformation ratio), VDP is the vapor pressure deficit (hPa), R is the total sum of rainfall during the growing period of the cultivated crop (mm, m^3), E_s is the seasonal soil evaporation (equal to 110 mm), and WR denotes the water reserves in the rooted soil zone (mm, m^3).

The main component of this equation is TE. Its value for wheat in Australia has been estimated at 20 kg grain mm^{-1} of water, with a maximum of 30 kg grain mm^{-1} [143]. However, this index depends on the amount of water available during the growing season; for example, as reported for spring triticale grown in a humid climate (Poland), the TE value ranged from 15 to 39 kg grain mm^{-1} [93]. This wide range clearly indicates the high sensitivity of this index to soil fertility and, consequently, to the effect of nutritional factors on plant growth and yield. Moreover, WLY cannot be regarded as a constant climatic value, as it largely depends on the amount of available water during the growing season for the currently cultivated crop. As shown in Figure 9, for maize, the WLY ranged from 8.52 t ha^{-1} in a year with a normal pattern of weather conditions to 5.68 t ha^{-1} in a year with drought. The obtained yields, despite the completely different course of weather, were relatively high. The main reason for this was soil fertility (sandy loam, naturally rich in available K and other nutrients). The main conclusions that can be drawn from this Figure are as follows:

(1) The content of available K (in the medium range) was enough to achieve the highest grain yield.
(2) The interaction of K and N was observed, regardless of the course of the weather.
(3) If the available K in the soil (being in the high range) is excessive, the yield will decrease.

The yield drop was accelerated by the increased dose of N_f. This unexpected effect (i.e., yield suppression) was due to the excessive accumulation of N in maize biomass before flowering, which resulted in a reduction in the number of kernels per cob [144,145].

The analysis of the partial factor productivity of the N_f index (PFP-N_f), for the presented case, is even more interesting. In 2001, the highest value of the index (109 kg grain kg N_f^{-1}) was recorded on a plot with high K level and fertilized with 100 kg N ha^{-1}. The highest yield, however, was achieved on a plot with a medium K availability range and with 140 kg N ha^{-1}, resulting in PFP-N_f of 100 kg grain kg N_f^{-1}. Both values are high, compared to the literature data [146]. In the presented case, it is worth discussing the rational choice of both N and K fertilizing systems. Raising the K soil fertility level to the high class resulted in a reduction in the dose of N_f by 40 kg ha^{-1}. It is necessary to take into account the fact that, on the field with the medium K level, the application of 140 kg N ha^{-1} increased the yield by 29%, when compared to the treatment with 100 kg N ha^{-1}.

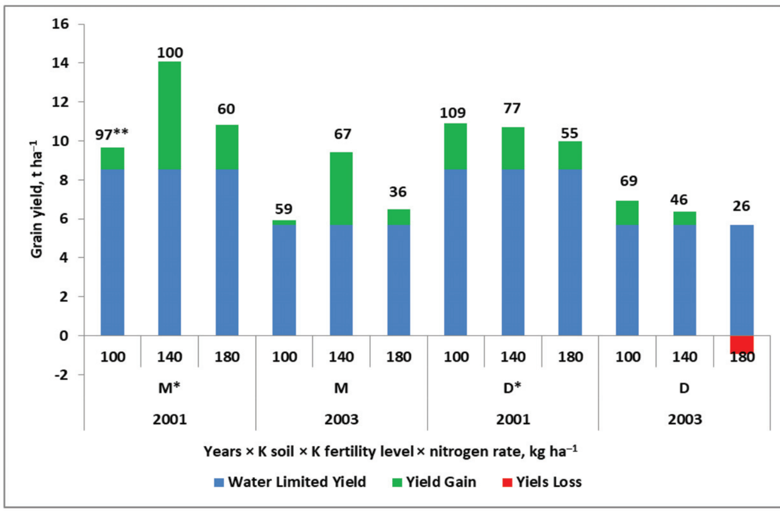

Figure 9. Effect of K soil fertility on maize yield in two years differing in water regime (modification based on [144]). Legend: * soil K fertility level: M, medium, D, high; ** Partial factor productivity of N_f (kg grain kg^{-1} N_f).

The WLY concept is a good tool for scientific studies. In agricultural practice, its use requires a set of data that is not typically readily available to the farmer. Moreover, this method does not explain the action of factors responsible for WUE. A proposed alternative method for determining Y_a is the concept of nitrogen gap (NG) [91]. The main components of this methodological approach for yield gap calculation are the N_f applied to the crop (which is known to the farmer) and the main yield. This data set may be enriched with other environmental and agronomic data that impact the NUE. The calculation procedure consists of the following steps:

$$\text{Partial Factor Productivity of } N_f: \quad PFP_{Nf} = \frac{Y}{N_f}\left(\text{kg kg}^{-1}\ N_f\right) \tag{11}$$

$$\text{Attainable maximum yield}: \quad Y_{attmax} = cPFP_{Nf} \cdot N_f\left(\text{t, kg ha}^{-1}\right) \tag{12}$$

$$\text{Yield Gap}: \quad YG = Y_{attmax} - Y_a\left(\text{t ha}^{-1}\right) \tag{13}$$

$$\text{Nitrogen Gap } (N_{uw}): \quad NG = \frac{YG}{cPFP_{Nf}}\left(\text{kg N ha}^{-1}\right) \tag{14}$$

where
N_f is the amount of applied fertilizer N (kg ha^{-1});
PFP-N_f is the partial factor productivity of N_f (kg grain/seeds, tubers, etc., per kg N_f);

Y_{attmax} is the maximum attainable yield (t ha^{-1});
*c*PFP-N_f is the average of the third quartile (Q3) set of PFP_{Nf} indices, arranged in ascending order (kg grain/seeds, tubers, etc., per kg N_f);
YG is the yield gap (t ha^{-1});
NG is the nitrogen gap (kg N ha^{-}).

The advantage of this method over the WLY is its simplicity in determining both Y_a and Y_{attmax} in a well-defined soil–climatic geographical area. The farmer, having access to data on environmental and agronomic production conditions (e.g., soil texture, soil pH, contents of basic nutrients, plant variety, level of plant protection, date of sowing) in their production region, can determine factors limiting the Y_{attmax}. The Y_a is a function of the formula:

$$Y_a = Y_{attmax} - YG \tag{15}$$

The NG values are required to produce a Y_{attmax} diagram, showing the distance between Y_a and Y_{attmax}. The detailed procedure for preparing the NG diagram has been described in recently published papers [147,148]. The distance Y_a for a specific field from Y_{attmax} can also be expressed by the fractional Y_a index (Y_{af}):

$$Y_{af} = \frac{Y_a}{Y_{attmax}} \tag{16}$$

A value of Y_{af} approaching 1.0 indicates sustainable management of applied N_f.

7.3. Factors Affecting N Fertilizer Use Efficiency

As shown in Equations 1 and 2, the Y_a of the cultivated plant is the result of the interaction between Y_{attmax} and efficiency of applied N_f (EN_f). In turn, the EN_f depends on the effectiveness of other factors that limit or increase the productivity of N_f.

The total number of factors which impact EN_f can be divided into five main groups:

(1) Farm organization and management;
(2) Agronomic factors (e.g., cropping sequence ≈ crop rotation, soil tillage, seed bed preparation, cultivar, sowing date, harvest date);
(3) Plant protection treatments, preventing yield reduction due to pathogens and pests;
(4) Fertilizing treatments aimed at the correction of soil fertility;
(5) Fertilizing treatments aimed at the in-season correction of the nutritional status of the grown plant.

The first group of production factors (i.e., organization and management of plant production processes on the farm) should not have a negative impact on the yield. In an economically well-run farm, the effectiveness of this group of factors should be at the level of 1.0—this is, after all, a basic prerequisite of the food production approach, known as the Sustainable Intensification of Agriculture (SIA) [14]. However, its implementation is only apparently easy. In fact, plant production on farms is under deep economic pressure [149]. A classic example is the method of plant cultivation. The economical decision to grow crops in monoculture, instead of crop rotation (a classical example is maize), may significantly reduce production costs, but a farmer must be aware of yield decreases [150]. There remains, however, uncertainty regarding the effectiveness of other production factors, which usually deteriorate. The decrease in yields of classic cereals grown under long-term monoculture is substantial. As shown in Figure 1, for winter rye, it can reach −20% under NPK treatment. The shortage of K resulted in a yield drop by 24% and that of P by 33%. There are also negative environmental effects to such an approach [151].

The farmer must effectively manage all agronomic factors and the health of the plant [61]. The efficiency of these factors, in accordance to the SIA concept, should be also set at 1.0. In this group, tillage and crop rotation are of particular importance for the available N pool and the uptake of its inorganic forms (N_{min}) by plants [152,153]. The main task of soil tillage is to mix plant residues, manure, and mineral fertilizers—especially those containing low-mobility nutrients (P, K)—into the topsoil. No less important is the loosening of deeper soil layers and elimination of the plow sole [154]. The main production goal of this set of agronomic treatments is to increase the potential of the currently grown plant to penetrate the subsoil with its roots. It cannot be considered as only a source of water, as it is an important source of both N_{min} and N-SNs. Under conditions unfavorable to plant growth, such as drought, the yield is largely determined by water and nutrient resources in the subsoil [155–157]. Unfortunately, the greatest weakness of the current methods for diagnosis of soil fertility and the resulting fertilization recommendations (apart from N) is a lack of methods for assessing the capacity of these resources and the availability of N-SNs. A simple technical and diagnostic solution is the use of extractants for N_{min} [158,159].

The succession of plants grown on a given field is highly important for the effective management of N. A classic, biologically documented pattern of plant succession is the Norfolk rotation, which has been known for about two centuries [54]. As shown in Figure, 1, the cultivation of winter rye, a crop considered by farmers to be tolerant to monoculture, resulted in the significant yield reduction, which was exacerbated by the lack of balance of N with other nutrients. The cultivation of winter wheat (WW) after cereals also leads to yield reduction [67,160]. As has been reported by Babulicova [160], the yield of WW following legume plant was 29% higher than that following cereals. The well-established crop sequence is based on the assumption that dicotyledonous and monocotyledonous crops should be cultivated alternately in successive years. The advantages of crop rotation, regarding the efficient use of N_f are [65,87,161,162]:

(1) The use of natural sources of N available on the field and farm. This leads to a reduction in the need for N_f.
(2) Biological subsoil amelioration by the strong root systems of dicots. Expected agronomic effects lead to:
    a. Mobilization of the soil nutrient resources (root exudates, mycorrhiza);
    b. Increased soil water capacity, resulting in better infiltration of rainwater;
    c. Increased exploration of the subsoil (i.e., growth of cereal roots in the root pores of dicots).
(3) A narrower C:N ratio in manure and residues of legumes. Both sources of organic matter have a positive effect on humus formation and content in the soil.
(4) The exploitation of soil nutrient resources within and in the soil profile is more sustainable, both qualitatively and quantitatively.

Crop rotation should not be considered by the farmer as a factor that decreases yield. Unfortunately, this is not the case, as has been evidenced in the scientific literature and agricultural practice. The key reasons for yield reduction due to wrongly planned crop succession (or even monoculture), to a great extent, are:

a. Insufficient N supply during the critical stages of yield formation, reducing the main components of the yield [67];
b. Disturbances in the processes of N transformation and uptake from soil [150];
c. Disturbances in the uptake of nutrients responsible for NUE (Figure 1; [163]);
d. Attack by pathogens, reducing the photosynthetic potential of the plant [164].

8. An Efficient System for Management of N-SNs—Principles of the Soil Fertility Clock

8.1. State of K and P Fertility Level and Food Production

The crop production potential of a single field in inherently related to its soil fertility level, presented as the depth of the humus profile and the content of available nutrients [165]. Fertile soil creates conditions for the build-up of a large (extensive) root system, which is crucial for the uptake of non-mobile nutrients, such as phosphorus and potassium [166].

The share of N, P, and K fertilizers in total nutrient uptake by cereals has been assessed as 33% for N, 16% for P, and 19% for K [167]. In China, as reported by Ren et al. [168], K fertilizer covers less than 20% (18.5%) of the total K in winter oilseed rape at harvest. Khan et al. [169], who studied 1400 field trials fertilized with K, did not observe any significant impact of the applied K (as KCl) on the yield of basic crops. According to MacDonald et al. [170], 29% of the world area of arable soils shows a deficit, while the remaining part shows a surplus of available P. The significant impact of P fertilizer can be revealed, as a rule, on soils with a low content of available P. The yield loss due to deficiency of P supply (P yield gap) to wheat is 22% (18–28%), 55% for maize (47–66%), and 26% for rice (18–46%). Moreover, the application of P fertilizers reduced this production gap to only 17% for wheat, 46% for maize, and 15% for rice [116]. At this point, it is necessary to ask whether the yield gap is actually due to a deficiency of available P, or the ineffectiveness of N_f due to the imbalance of P and other nutrients.

The key question to be formulated is: what is the appropriate level—or rather, the critical range—of N-SNs content in the soil? In the light of the facts presented above, classic P and K management strategies require significant modifications. The basis for these required corrections is the fact that the plants are grown in a cropping sequence determined by the economic goals of the farm. Not all modern crop sequences follow rational principles (i.e., biologically based crop plant succession) [54,171–173]. It has been well-documented, in millions of scientific articles, that the deviation of a particular cropping sequence from biological rules leads to a decrease in yield. The classic example is the Rothamsted long-term experiment with winter wheat [67]. Wheat followed directly by wheat or grown in monoculture yielded a significantly lower level, compared to that following dicotyledonous plants. Second, the maximum grain yield achieved under non-optimal rotation was, in this example, both lower and, at the same time, required a higher N_f rate. These results clearly indicate lower unit N_f productivity due to N immobilization, disturbance in uptake of N-SNs, and stronger pressure by pathogens [150,174–176].

The soil resources of P and K are the main source of nutrients for the cultivated crop. Over-exploitation of available pools of these nutrients leads to the degradation of soil fertility, subsequently resulting in the lower N_f productivity. Moreover, these processes create a multi-level risk, for the yield, farm economics, and the environment (Photos 2 and 3; [177]). The productivity of a particular field is determined by the level of soil fertility, conditioned by water capacity and the content of available N-SNs in the rooting zone of the currently cultivated crop [153,154,157]. At present, the subsoil resources of N-SNs are not included in typical soil fertility status diagnostic procedures. Moreover, there is a frequently presented opinion regarding their low significance for the plant growth and yield [178]. In the light of the published data, the share of P and K from fertilizers for crop plant nutrition is of secondary importance for the maintenance and synchronization of plant needs and nutrient supply from the soil. The logical conclusion to be drawn from these facts is unambiguous: the farmer's goal for effective management of N_f is not to use P and K fertilizers or other carriers of these nutrients for direct feeding of the plant, but to coordinate the soil fertility level to maximize N_f use efficiency.

8.2. Management of Soil Fertility—Oriented to Cropping Sequence

Soil productivity is the ability of arable soil to provide the currently grown plant with air, water, and nutrients in the required amounts, mutual proportions, and ratios, ensuring full expression and development of the yield components [179]. This general property of arable soils has been indicated as one of the most important objectives listed in the Sustainable Development Goals (SDGs) by the United Nations in the 2030 Agenda for Sustainable Development [180]. This global goal can be achieved, but only through two targeted actions. The first is oriented towards stopping the degradation of soil fertility. This action refers to the world regions where soil fertility has been drastically reduced [10,177]. The second requires a significant correction in N-SN management strategies in areas of the world with advanced crop plant productivity. The so-called Old Agricultural Areas of the world will be decisive for food supply to the growing human population in the coming decades.

At present, two main concepts dominate in soil fertility management. The first—called the maintenance approach—is based on the assumption that the main goal of N-SNs is necessary to maintain the content of available nutrients at a certain level, allowing crop growth and yield. Three phases of soil fertility build-up can be distinguished using this approach: (i) build-up, (ii) maintenance, and (iii) draw-down [181]. In practice, the recommended rates of nutrients increase with the size of the gap between the maintenance level and the actual soil fertility status for a given nutrient. In most countries, using this fertilization approach, the nutrient doses recommended by agrochemical testing laboratories are consistent with the state of its deficiency. A classic example is the K recommendation in China for WOSR yielding at 3.75 t ha^{-1} [168]. The decreasing content of available K (NH_4OAc-K extraction method) resulted in increasing the dose of applied K from 232 kg ha^{-1} at low K range to 50 kg ha^{-1} at high K range. The second approach, called sufficiency ranges, is based on the required (i.e., sufficient) level of the given nutrient for the respective crop [181].

These two fertilization strategies are based on the assumption that low-mobility nutrients are as effective as nitrate nitrogen [147]. The coefficient of effective diffusion for the N form is 2.7×10^{-1} cm^2 s^{-1}. In comparison, this index for K^+ and $NH_4{}^+$ ions are about 100-fold lower (1–28 $\times 10^{-8}$ and 6.1×10^{-8} cm^2 s^{-1}, respectively). For $H_2PO_4{}^-$ ions, this index is 10,000-fold lower, compared to nitrates [182,183]. The differences in the uptake rates of N and K are shown for sugar beets in Figure 7. Within 7 days of sugar beet growth in July, the N-NO_3 resources, but not K, were completely depleted (100%) to a depth of 1.8 m. For K, this level of depletion was reached at a depth of 0.5 m. The most intensive uptake of both nutrients took place in the soil layer (0.0–0.6 m). There are two main conclusions to be drawn from Figure 10:

(1) The nutrients are exploited from the whole root zone of the currently grown crop plant;
(2) The faster uptake of nitrates than K^+ by the fibrous roots of sugar beet means that a significant part of the low-mobility nutrients in the soil will not be taken up (i.e., not used up in the growing season).

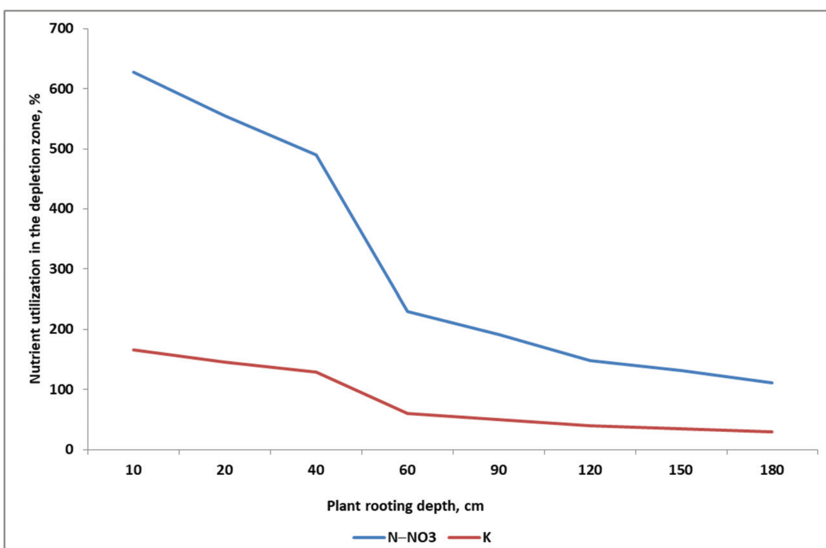

Figure 10. Degree of nitrate and potassium utilization in the soil during the maximum stage of K accumulation by sugar beets (based on [74]).

The classic concepts of N-SNs do not take into account two crucial facts; that crop plants differ in their sensitivity to the supply of N-SNs:

a. During the growing season;

b. In the course of crop rotation.

The efficient management of N-SNs in a soil/plant system should be based on the following principles of crop production:

(1) Annual crop plants should be cultivated in a fixed sequence (i.e., the crop rotation).
(2) Cereals have, as a rule, lower requirements for K, but higher requirements for P, compared to non-cereal crops.
(3) Dicotyledonous plants have higher requirements for K than cereals.
(4) The architecture of the root system of cereals is, as a rule, extensive compared to dicotyledonous plants. Consequently, cereals are less sensitive to the level of P and K fertility.
(5) The distribution of low-mobility nutrients varies with depth.
(6) Plants during the growing season differ significantly in the critical stages of nutrient requirement:
    a. Seed crops show critical periods, in terms of P requirements, during the vegetative (minor one) and reproductive (main one) periods of growth;
    b. All crops are sensitive to K during the linear phase of growth.
(7) The critical period for N requirements by a seed crop is related to stages of seed/grain density formation.
(8) The key yield-forming function of P in all crops is to accelerate the early rate of the plant growth.
(9) The exploitation of P resources by seed crops, accumulated in vegetative parts before flowering, depends on seed/grain density.
(10) The yield-forming function of K in
    a. Seed crops is to strengthen N action;
    b. Dicotyledonous crops is acceleration of the early rate of the plant growth (mostly up to the rosette stage).

All of these points should be taken into account by the farmer during the process of development of the fertilization system.

Soil Fertility Clock (SFC) is an approach based on three assumptions which are key to the effective management of N_f:

(1) Critical soil fertility is the value or range of a soil nutrient's content that is sufficient to provide it in the appropriate amount to the plant most sensitive to its supply in a given crop rotation.
(2) Other, non-sensitive plants in the given crop rotation create the necessary time-frame for recovery of its original critical content.
(3) The content of a specific nutrient cannot be a limiting factor in N uptake and utilization for any crop grown. Its fractional use efficiency, regardless of the actual plant in crop rotation, is $\leq$1.0.

The SFC concept is visualized in graphical abstract and explained in Figure 11 and Table 1. The critical K level for oilseed rape or any other dicotyledonous crop creates favorable conditions for the succeeding crop; that is, cereals, and most often wheat. The K level will still be high enough to cover the K requirement of wheat. A third crop in a certain cropping sequence—for example, maize—requires the farmer's attention to adjust the K content. This is necessary only if the K content drops below the medium level. This is very probable when harvest residues are removed from the field (Table 1). The critical period of K correction, which must be oriented toward the so-called crop rotation critical K level is, in the discussed case, the spring barley growing season. This is a key term in the agronomic clock for determining both the level of K in the soil and determining the fertilization needs for the plant sequence spring barley $\rightarrow$ winter oil-seed rape. Managing P in crop rotation is a bit more complicated. It requires, regardless of the grown crop, the use of a starting dose of P fertilizer. Basic P fertilization complies with the principles presented for K. An additional component of an effective system regarding the full set of nutrients is foliar fertilization. This method allows for the correction of plant nutritional status—in fact, N action—but only at stages preceding the critical stages of yield formation.

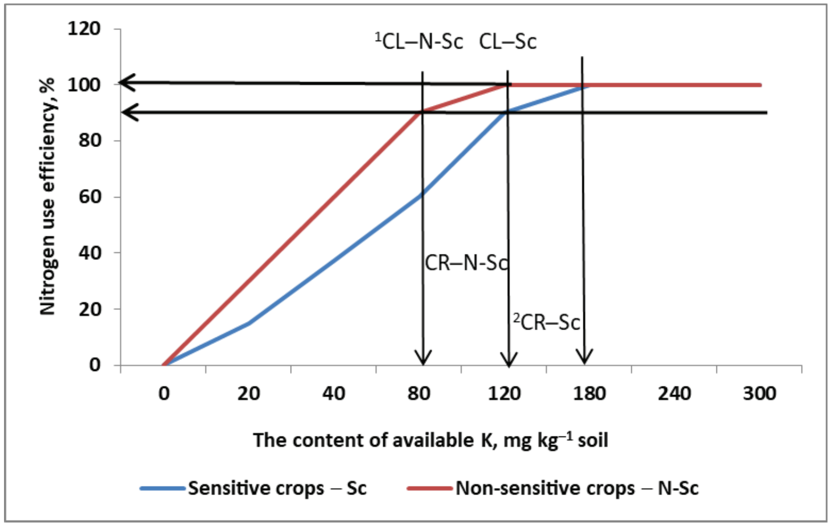

Figure 11. The crop rotation conceptual approach for the sufficient K level and range on sandy loam for sensitive and non-sensitive K plants. Legend: [1] CL–N-Sc, CL–Sc, CR–N-Sc, [2] CR–Sc: critical level and critical range for non-sensitive and sensitive plants to the content of available K (determined by the Egner–Riehm method).

Table 1. Phosphorus and potassium balance in four-course rotation with winter oilseed rape. under full use of straw [1,*], t ha^{-1}.

Crop Rotation	Nutrients			
	Phosphorus, P_2O_5		Potassium K_2O	
	Demand	Full Recycling	Demand	Full Recycling
Winter oilseed rape, 3.5 t ha^{-1}				
-seeds	70	-	35	-
-straw	40	20 **	200	180 **
Winter wheat, 7.0 t ha^{-1}				
-grain	55	-	35	-
-straw	25	12	120	110
Maize, 8.0 t ha^{-1}				
-grain	60	-	40	-
-straw	30	15	160	145
Spring barley, 5.0 t ha^{-1}				
-grain	45	-	25	-
-straw	10	5	110	100
Total sum	335	53	725	535
Balance	−282		−190	
Total demand	**282**		**190**	
Partial demand, kg ha^{-1} year^{-1}	**70.5**		**47.5**	

[1] Simulation based on authors own data; * average range of soil fertility with P and K; ** recovery of P and K from straw during four-course rotation, phosphorus −50%, potassium −90%.

9. Conclusions

Many factors directly and indirectly affect the yield of plants grown on the farm. The dominant one is N, which determines the dynamics of plant growth, partitioning of assimilates between the plant's organs, the expression of yield components, and consequently the yield. Currently, effective plant production is based on the use of N_f. The first step in an efficient management of N_f is to determine the maximum attainable yield of crops grown on the farm. This yield category is defined by the basic environmental factors, i.e., climate and soil fertility, but at the same time it is strongly modified by agronomic factors (crop rotation, soil tillage, plant protection). However, the most important of these factors is the N_f dose. The target yield can be achieved as long as the efficiency of N_f approaches 1.0, but at the same time the N_f, regardless of the dose, does not reduce the yield. The synchronization of N demand, which varies in the plant's life cycle with the rate of its uptake (in fact, nitrate) from the soil, is not dependent on its soil resources. This condition is met, but only when it is balanced with the supply of other nutrients (nitrogen-supporting nutrients; N-SNs). This, it can assumed that effective control of N_f efficiency does not only depend on its applied dose. The yield of high-yielding crops determines the interaction efficiency of N $\times$ P and N $\times$ K. The requirements of crop plants for P and K during the growing season are time-separated. The period of intensive biomass growth is a critical stage in the sensitivity of the plant to the supply of K. In seed plants, the N $\times$ K interaction predefines the development of yield components. The sensitivity of plants to the supply of P is revealed both in the early stages of growth and in the phase of yield realization. The second phase is crucial for seed plants. Phosphorus deficiency results in poor development of fruits, grains and seeds. A deficiency of both nutrients in the soil during the critical stages of yield formation results in both a decreased N_f efficiency, and consequently, a lower yield. The basic production unit on a farm is the field, on which plants are grown in a specific time-sequence, known as crop rotation. The condition for achieving the required level of N_f ($\leq$1.0) efficiency is the high effectiveness of other production factors, which is to be set at $\leq$1.0. The operational basis of an effective control of N_f efficiency is the content of P and K in the soil, which should be oriented to cover the requirements of the most sensitive plant in a well-defined crop rotation. Thus, the main goal of P and K application to the soil is to restore their content in the topsoil to the level required by the most sensitive crop in a given crop rotation. The other crops grown in this cropping sequence provide the time-frame to actively control the distance between the current P and K content from the required critical ranges.

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Review

Fertilizers and Fertilization Strategies Mitigating Soil Factors Constraining Efficiency of Nitrogen in Plant Production

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Abstract: Fertilizer Use Efficiency (FUE) is a measure of the potential of an applied fertilizer to increase its impact on the uptake and utilization of nitrogen (N) present in the soil/plant system. The productivity of N depends on the supply of those nutrients in a well-defined stage of yield formation that are decisive for its uptake and utilization. Traditionally, plant nutritional status is evaluated by using chemical methods. However, nowadays, to correct fertilizer doses, the absorption and reflection of solar radiation is used. Fertilization efficiency can be increased not only by adjusting the fertilizer dose to the plant's requirements, but also by removing all of the soil factors that constrain nutrient uptake and their transport from soil to root surface. Among them, soil compaction and pH are relatively easy to correct. The goal of new the formulas of N fertilizers is to increase the availability of N by synchronization of its release with the plant demand. The aim of non-nitrogenous fertilizers is to increase the availability of nutrients that control the effectiveness of N present in the soil/plant system. A wide range of actions is required to reduce the amount of N which can pollute ecosystems adjacent to fields.

Keywords: crop growth rate; fertilizer market; nitrogen use efficiency; nitrogen gap; nutrient uptake; partial factor productivity; root architecture

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1. Fertilizer Use Efficiency—A Real Farming Practice

1.1. Nitrogen Gap and the Maximum Attainable Yield

A farmer needs to recognize production boundaries in order to develop an effective production program for each of the crops grown on the farm. The key to the sound management of production processes is a knowledge of the maximum yield that can be achieved in a production area with a well-defined climate and soils. The actual yield (Y_a) of a currently cultivated crop may be simply presented as the difference between the maximum attainable yield (Y_{attmax}) and the yield gap (YG). The relationship between these terms may be expressed as the formula:

$$Y_a = Y_{attmax} - YG \tag{1}$$

Y_a is a real, harvested yield in the current growing season under actual environmental, agronomic and management practice on the farm. To define the Y_{attmax} of this crop, two conditions must be fulfilled. The first concerns a strictly defined climatic area and the dominating, i.e., standard, weather conditions [1,2]. The second necessary condition is the level of soil fertility, agronomic conditions and management of the production processes on the farm. These factors modify the Y_{attmax} of the grown crop [3,4]. All of these factors must be oriented towards optimizing the supply of nutrients to that particular crop only [5]. The YG is a measure of the ineffectiveness of production factors, in fact expressed in the ineffectiveness of fertilizer nitrogen (N_f), or available N present in the soil/plant system during the growing season of the currently grown crop [6]. The basic and at the same time simplest method for calculating both components of the Y_a formula is to use the efficiency index of N_f known as the Partial Factor Productivity of Fertilizer N, PFP_{Nf} [7]. Considering both the yield and the environmental aspects of the on-farm production process, the farmer's goal should not be to determine the YG, but rather the ineffectiveness of the applied N_f. The quantitative expression of N inefficiency is the nitrogen gap (NG) [8]. In fact, two sets of data are needed to determine both the YG and the NG, i.e., (i) the actual yield harvested by the farmer, and (ii) the amount of applied N_f. The calculation procedure consists of a set of formulas:

Partial Factor Productivity of N_f:

$$PFP_{Nf} = \frac{Ya}{N_f}\left(kg\ kg^{-1}\ N_f\right) \quad (2)$$

Attainable, maximum yield:

$$Y_{attmax} = cPFP_{Nf} \times N_f\left(t\ or\ kg\ ha^{-1}\right) \quad (3)$$

Yield Gap:

$$YG = Y_{attmax} - Y_a\ \left(t\ ha^{-1}\right) \quad (4)$$

Nitrogen Gap:

$$NG = \frac{YG}{cPFP_{Nf}}\left(kg\ N\ ha^{-1}\right) \quad (5)$$

where: PFP_{Nf}—partial factor productivity of N_f, kg grain/seeds, tubers etc. per kg N_f; Y_a—actual yield of a currently grown crop, t ha^{-1}; N_f—the amount of applied fertilizer N, kg ha^{-1}; Y_{attmax}—the maximum attainable yield, t ha^{-1}; $cPFP_{Nf}$—the average of the third quartile (Q3) of the set of PFP_{Nf} indices arranged in ascending order, kg grain/seeds, tubers etc. per kg N_f; YG—yield gap, t ha^{-1}; NG—nitrogen gap, kg ha^{-1} of N.

The NG calculation is important for the farmer for at least three areas of his production activity: (i) the determination of Y_{attmax}, which determines not only the maximum yield for the production area, but also determines the potential requirements of the cultivated crop for N; (ii) the identification of hotspots in N management for a given crop, including an inadequate supply of nutrients other than N; (iii) the set of actions needed to improve the level of soil fertility for a given crop.

The data on NG is used to construct a diagram of the impact of the NG change on trends in actual and maximum yields (Figure 1). The target of the NG construction is to find the maximum attainable yield (Y_{attmax}) for the geographical area of the farm operation. The Y_{attmax} value is determined by the intersection of Y_{max} and Y_a linear regression models. In this specific case, representing 16 fields located in a small region of central-western Poland, the weather and soil conditions are stable. Y_{attmax} for winter wheat reached 7.99 t ha^{-1}. Moreover, both Y_a and Y_{attmax} showed significant variability in the amount of *notworkable* N_f during the growing season. The course of both models indicates a surplus of N_f on fields No. 13 and 10 as the main reason for its lower use efficiency. The maximum YG on field No. 13 reached 3.729 t ha^{-1}, i.e., it constituted 47% of the actual yield. The diagnostic goal of the NG diagram construction is to identify the key factors responsible for YG appearance as a result of N_f inefficiency. The ranges for the evaluation of the effect of any production factor were constructed using a clear scale: low, medium, high, which were in special cases underlined by "very". The use of this scale to assess the production effect of N_f is shown in Table S1 (Supplementary Material).

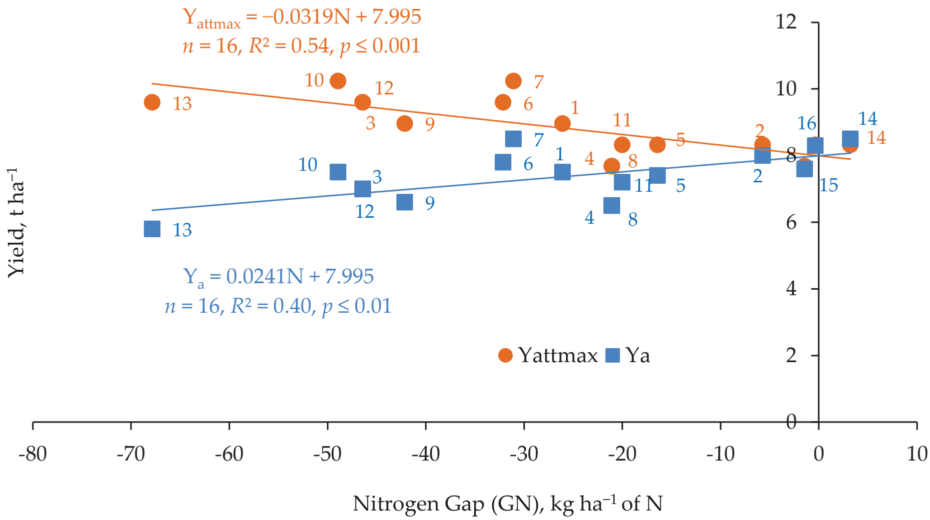

Figure 1. Diagram of yield trends in response to the nitrogen gap (NG) change. Example for winter wheat (based on Grzebisz and Łukowiak [8]). Key: Y_{attmax}—maximum attainable yield; Y_a—actual yield; 1–16 are the field numbers.

1.2. Fertilizer Use Efficiency—FUE

The term Fertilizer Use Efficiency—FUE is not new. It has been widely used for decades but has become widespread recently thanks to the use of the FUE indexes to assess the global productivity of NPK fertilizers [7,9]. The productivity of nutrients applied in fertilizers can be estimated by the same formula as shown in Equation (2) for fertilizer N. Another methodological way for FUE determination is to use a set of indices used in field experiments such as Apparent Nutrient Efficiency (ANeE) and/or Apparent Nutrient Recovery (ANuR):

$$ANuE = \frac{Y_f - Y_c}{N_r} \quad (6)$$

$$ANuR = \frac{Nu_f - Nu_c}{N_r} \quad (7)$$

where: ANuE—Apparent Nutrient Efficiency, kg yield kg^{-1} nutrient applied; ANuR—Apparent Nutrient Recovery, %; Y_f, Y_c—yield on a plot with and without fertilizer, t or kg ha^{-1}; N_r—the rate of a nutrient applied as fertilizer, kg or g ha^{-1}; Nu_f, Nu_c —the uptake of a tested nutrient on a plot with and without fertilizer, kg, g ha^{-1}.

The recorded values of ANuE and ANuR usually show a decreasing trend, with an increase in the rate of the nutrient applied as fertilizer, which is satisfactory for the researcher. Moreover, the values obtained have a tendency opposite to the soil fertility indexes for a given nutrient [7]. It simply means that FUE is highly dependent on the current soil fertility level, which the farmer needs to know. However, the main disadvantage of these two indices is that the farmer does not have a control plot to assess the actual nutrient productivity in the applied fertilizers. The values of the ANuR indices, evaluated on the global scale, are low and amount to 40–65% for N, 15–25% for P, and 30–50% for K used in fertilizers [9]. At this point it is necessary to pose the question, what is the main source of nutrients for the currently grown crop?

The productivity of nutrients taken up by the crop during one growing season can also be estimated by the partial nutrient balance (PNB) method:

$$NuE = \frac{Nu_t}{Nu_f} \times 100\% \quad (8)$$

where: NuE—Nutrient uptake Efficiency, kg kg^{-1}; Nu_t—the uptake of a tested nutrient, kg or g ha^{-1}; Nu_f—the rate of a nutrient applied as fertilizer, kg, g ha^{-1}.

The efficiency of N, P, and K using this method show much higher values or even a surplus of nutrients [10]. The low efficiency of nutrients using the differential methods, but high yield indirectly indicates that the main source of nutrients for crops grown in one growing season is soil [11].

The main problem is the assessment of the production role of nitrogen, which plants take in in two distinct inorganic forms, i.e., as nitrate ($NO_3{}^{-1}$) and ammonium ($NH_4{}^{+}$) [12]. Nitrates affect plant growth in many ways, inducing plant morphology, physiology through hormones and finally metabolism through their influence on the production of organic acids [13–15]. Plants fed with nitrates, compared to ammonium, show a high growth rate, which results in higher yields [16]. The above-identified aspects of the impact of N on plants are fully supported by field experiments and agricultural practice [17,18]. As shown in Figure 2, the yields of winter wheat grown on the control plot (non-fertilized) and on the plots fertilized with K, P in the same way since 1957, did not show large differences. The average yield for these three objects of 4.38 ± 0.14 t ha^{-1}, can be considered as high. The primary reason for such a high yield, despite the lack of N fertilization, was alfalfa as a forecrop. The use of 90 kg N ha^{-1} increased the yield by 1.94 t ha^{-1}. The same level of yields was also recorded for the NP and NK plots. The lack of response to the P or K application clearly emphasizes the importance of these two nutrients for plant growth and yield. This conclusion was fully confirmed by the yield achieved on the NPK plot. Even more important is the fact that N use efficiency (NUE) increased by 10–13%, compared to incomplete fertilization treatments. The observed interaction was even more important for P use efficiency (PUE), which in the NPK plot increased by 9% and by 73% compared to NP and P treatments, respectively. The same trend was observed for potassium. The importance of the N $\times$ PK interaction on the productivity of N_f is observed for all crops, regardless of the world region [17,19,20]. The complex effect of N on plant growth and yielding clearly indicates the superior function of N in crop production. It can, therefore, be concluded that the production efficiency of nutrients, applied as mineral fertilizers, can be mainly evaluated through their impact on NUE. Thus, the search for indicators of productivity or efficiency for other nutrients is pointless. This is well presented in the analysis of the causes of the NG (Table S1).

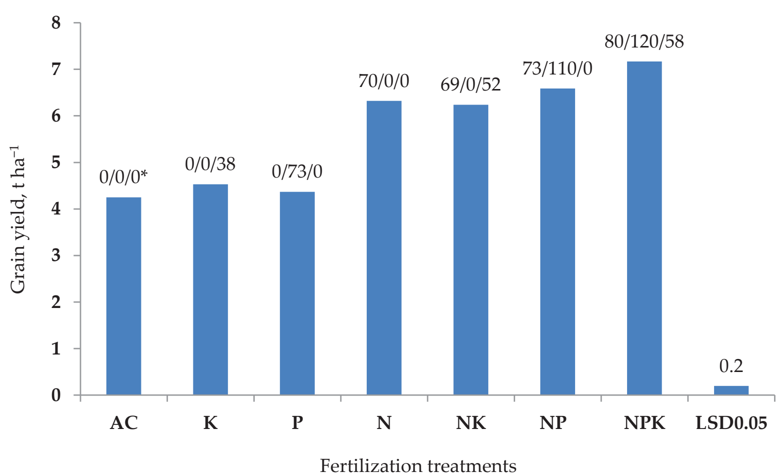

Figure 2. Effect of long-term differentiated fertilization on yield of winter wheat, mean of 2005–2008 years (own projection based on Blecharczyk et al. [17]). Key: AC—absolute control; K, P, N—experimental trials since 1957; $LSD_{0.05}$—Least Significant Difference; 0/0/0*—respective values of nitrogen, phosphorus, and potassium use efficiency.

1.3. Factors Affecting Fertilizer Use Efficiency

Fertilizer use efficiency is the result of a series of interactions between plant genotype and environment, including both abiotic and biotic factors. Full recognition of these factors is the basis for proper fertilization of plants in farming practice, aimed at maximizing the FUE values. The soil is both the growth environment for plants and their main reservoir of water and nutrients. Hence, the impact of soil factors on nutrient uptake and FUE should be considered at the level of several groups of phenomena and processes (Figure 3).

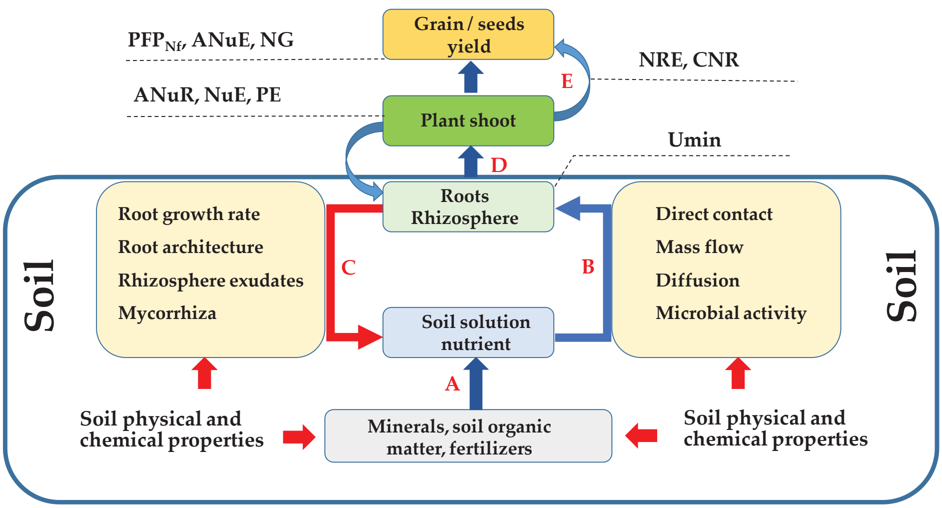

Figure 3. Fertilizer Use Effectiveness (FUE) indices in response to soil physical and chemical properties and processes responsible for nutrient uptake: (**A**) release of nutrients from solid phase; (**B**) processes of nutrient transport from the soil to the root surface; (**C**) the plant's physiological response to conditions of nutrient supply; (**D**) processes of nutrient transportation to the plant shoot; (**E**) nutrient remobilization and transfer into grain/seeds. Blue arrows—transport processes; red arrows—influencing and feedback responses. FUE indices explanations: PFP_{Nf} —partial factor productivity of nitrogen; ANuE—apparent nutrient efficiency; NG—nitrogen gap; NRE—nitrogen remobilization efficiency; CNR—contribution of remobilized N to grain; ANuR—apparent nutrient recovery; NuE—nutrient uptake efficiency; PE—physiological N efficiency; U_{min}—minimum uptake of a nutrient for the maximum rate of plant growth.

In the first group (A) all of the factors, both abiotic and biotic, that lead to the release of nutrients from their solid phase in the soil to their solution phase should be analyzed. The next group of factors (B) is concerned with the processes of transporting nutrients from the soil to the root surface. The third group (C) of factors influencing FUE concerns plant responses manifested by changes in architecture and root growth rate. This group of factors, also related to plant activity, should consider the composition of the root exudates in the plant root—mycorrhizal system. For the assessment of the effectiveness of fertilizer application, the processes taking place in the plant itself, related to transport, assimilation in the aboveground mass (D), as well as remobilization of components and their transfer from the vegetative parts to the generative crop (E), are also important.

2. Factors Affecting Nutrient Uptake

2.1. Plant Growth and Nutrient Requirement

A major challenge for the farmer is to synchronize the crop plant requirement for nutrients with their supply from both soil and applied fertilizers. The term synchronization refers to the amount of a nutrient that must be taken up by the crop at a certain stage of its growth as a prerequisite for a development of yield components. The expected degree of a given yield component formation depends on the growth rate of the crop, which in turn depends on the supply of N. For example, the critical stage of yield formation by winter oilseed rape (WOSR) reveals itself at the phase of inflorescence development (BBCH 50–59; coding system of growth stages, abbreviation in German: Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie [21]). As shown in Figure 4, WOSR fertilized with N as ammonium nitrate (AN) in two equal rates of 80 kg N ha^{-1} applied at BBCH 22 (spring restart of WOSR) and BBCH 30/31 reached the maximum growth rate (CGR_{max}, 20.4 g m^{-2} day^{-1}) at full flowering. This value was the prerequisite of both the highest yield and the lowest, in its year-to-year variation (coefficient of variation, CV of 5.6%).

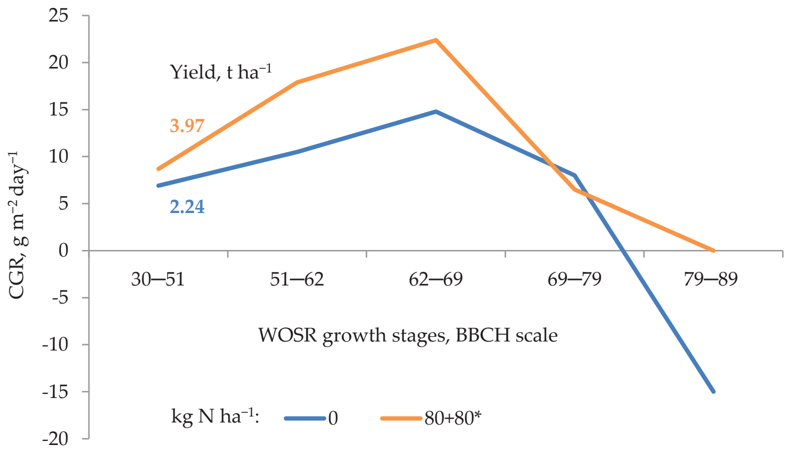

Figure 4. Crop growth rate (CGR) of winter oilseed rape (WOSR) during the growing season as affected by nitrogen fertilizer (based on Barłóg and Grzebisz [22]). Key: CGR—crop growth rate, N–0—absolute control, N–80 + 80*–N rate of 160 kg N ha^{-1} applied at the onset of the growing season restart in Spring; * ammonium nitrate; 30, 51, 62, 69, 79, 89—WOSR growth stages in BBCH scale.

For comparison, plants fertilized with the same N dose, but applied as calcium ammonium-nitrate (CAN), yielded on average at the same level, but showed much higher year-to-year variability. The main reason was a slightly lower CGR_{max} (19.1 g m^{-2} day^{-1}), resulting in a higher CV (16%). Moreover, plants fertilized with AN reached the maximum N accumulation at the full flowering stage (BBCH 65), while those fertilized with CAN much later, i.e., at the beginning of pod growth (BBCH 71). The observed delay was due to the excessive growth of secondary branches, which is not always coordinated with higher yield [23]. The lower yield on the N control plot was mainly due to a significantly lower rate of dry matter accumulation, which resulted in a much worse status of the yield components at maturity. The existing relationship between nutrient uptake by a plant and its growth rate can be summarized by the equation [24]:

$$U_{min} = C_c \times \frac{dW}{dt} \times \frac{1}{w} \times \frac{W}{2\pi rL} \text{ or } U_{min} = C_c \times RGR \times \frac{W}{2\pi rL} \quad (9)$$

where: U_{min}—minimum uptake of a nutrient for the maximum rate of plant growth, g or kg $plant^{-1}$ or unit area; C_c—critical concentration of a nutrient in a plant, g, mg kg^{-1} DW; W—aboveground biomass of a plant, g or kg DW; r—root diameter, mm or cm; L—root length, cm or m; 2πrL—root surface area, mm^2 or cm^2 or m^{-2}; $\frac{dW}{dt} \times \frac{1}{W}$—the relative growth rate of a plant, RGR, g g^{-1} t^{-1}; t—time: day or year.

This equation clearly shows that the minimum amount of a given nutrient taken up by a plant over a specific period of time is necessary to maintain its critical concentration in plant tissues, determining the plant's optimum growth rate. In the numerator of the equation, apart from the nutrient concentration, is the plant biomass, determined by two factors, i.e., the period duration (t—time) and the root surface area, as the denominator.

The first challenge for the farmer in exploiting the yielding potential of the grown crop is to recognize the critical stage(s) of yield formation, or more precisely, the formation of yield components. Plant crop development is usually described on a 100-point scale (stages), divided into 10 phases [25]. Farmers need to know this scale to control the development of yield components. However, its use by the farmer for precise fertilization requires identifying those stages, which are crucial for the development of the main yield component. The degree of its development is closely related to the crop biomass, which is described by the sigmoid crop growth model [26]. The accumulation of crop biomass during the growing season, based on this model, shows variable growth rates in different phases, which fits with exponential, linear, quadratic or linear-plateau regression models (Figure 5). This trait can then be used to determine the three growth mega-phases of crops [8,27]:

1. Exponential → Crop Foundation Period—CFP;
2. Linear → Yield Formation Period—YFP;
3. Quadratic or linear plateau → Yield Realization Period—YRP.

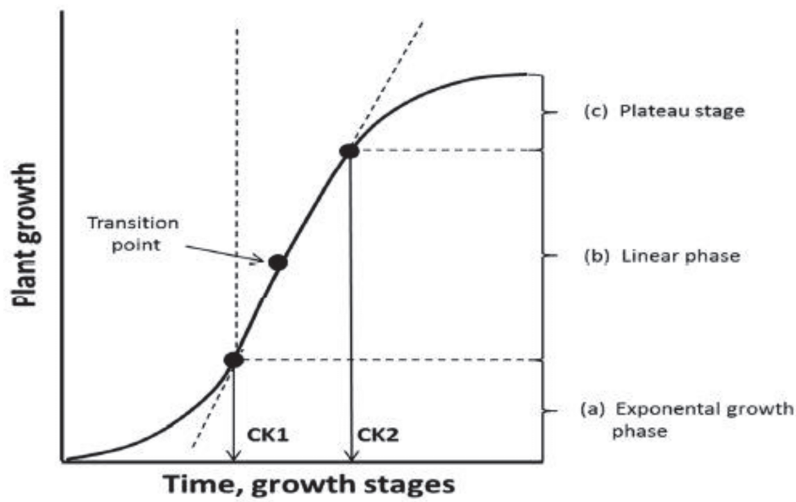

Figure 5. A conceptual pattern of dry matter accumulation by a typical seed/grain crop. Key: CK1, CK2—cardinal stage 1 and 2, respectively [8].

The first mega-phase refers to all crops, but the last one only to seed plants. The intersection points of CFP and YFP as the first pair, and YFP and YRP as the second, termed as cardinal knots (CKs), are two crucial points of the crop yield development [8]. CK1 is the change point at which the crop changes its rate of dry matter accumulation from the exponential to the linear model [26]. CKs are used by farmers as diagnostic steps to assess the crop nutritional status. CK1 is a crucial point at which to correct the nutritional status of all crop plants, regardless of the species [28]. In the case of cereals, CK1 refers to the borderline of tillering and the beginning of the stem elongation phase (BBCH 29–31). For dicots, this cardinal knot is related to the rosette stage. A classic example is winter oilseed rape ([25]; Figure 6). The critical nutrient concentration specified at CK1 is important, mainly for correcting the N status of the currently grown plant. For most crops, CK1 is the date of the maximum relative growth rate (RGR) of the crop. A classic example is maize. As shown in Figure 7, maize reached the maximum RGR on the 48th day after sowing (BBCH 15 to 17) and then its value decreased with increasing maize biomass. This particular period of maize growth is associated with the appearance of inflorescences [29,30]. Thus, the date when the plant reaches its maximum RGR defines the first cardinal phase of yield formation by the crop, i.e., CK1.

(**a**)

(**b**)

Figure 6. The Cardinal Stage 1 (CK1): winter wheat (monocot) (**a**) and winter oilseed rape (dicot) (**b**). Photos by W. Grzebisz.

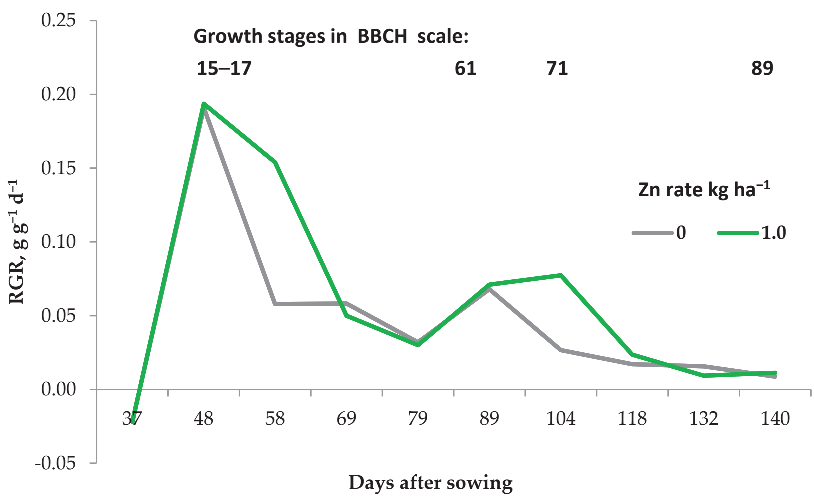

Figure 7. Relative growth rate (RGR) of maize during the growing season in response to foliar zinc (Zn) application (based on Grzebisz et al. [31]—modified).

Moreover, as shown in Figure 7, the zinc (Zn) foliar treated maize maintained, more strictly extended the duration of the RGR peak. As a consequence of the prolonged biomass growth at BBCH 15–17, a second RGR peak, but much smaller, appeared during flowering. The yield increases due to zinc application before the CK1 resulted in a yield higher by 1.49 t ha^{-1}. The partial factor of N productivity (PFP_{Nf}) increased from 66.7 to 79.3 kg grain per kg of N_f. The direct reason for the yield increase was the uptake of an N increase of 46.4 kg ha^{-1} [31]. The given example clearly indicates that the use of macronutrient fertilizers requires the precise diagnosis of the critical phase (s) of yield formation by the crop.

The second cardinal phase (CK2) is very well-defined for seed crops. This stage proceeds the date of flowering (Figure 8). For some crops, their nutritional status at CK2 can be used to forecast the yield. A classic example is maize. The nutrient content at this stage in the cob leaf is used to indicate the nutritional status of maize and delivers a highly reliable yield prognosis [32,33]. The same rule is observed for winter oilseed rape. The content of nutrients in leaves at flowering can be used to forecast the seed yield [34]. This relationship explains the opinion of Schulte auf'm Erley et al. [21] on the importance of the inflorescence phase in winter oilseed rape for the yield. However, the latest that the N dressing can be conducted is at the rosette stage [35].

(**a**) (**b**)

Figure 8. The Cardinal Stage 2 (CK2): winter rye (monocot) (**a**) and winter oilseed rape (dicot) (**b**). Photos by W. Grzebisz.

Nitrogen fertilization in cereals, to meet the requirements at CK2, should be conducted in the period between the date of the growth rate change (transition point) and flowering (Figure 5). In fact, in cereals, the last dose of N is applied at the end of the stem elongation phase. This phase precedes the period of the highest rate of ear growth, i.e., booting, which is responsible for the number of grains per unit area [36,37]. A separate case is bread wheat, where the last dose of N is used during the heading stage. The main goal is to increase the protein content in the grain [38].

A relevant and crucial component of nutritional crop status evaluation is a well-defined range of nutrient concentration in indicative plant parts and the relationships between them. Theoretically, there are some sophisticated methods for crop nutritional status assessment. The most commonly used are DRIS (Diagnosis and Recommendation Integrated System) and CND (Compositional Nutrient Diagnosis) [39,40]. In practice, farmers use, the sufficient ranges (SR) method to gain a quick evaluation of the crop nutritional status [41]. The biggest disadvantage of the SR method is the need for a large data set that is required for the calibration of the established ranges [42]. Moreover, most of the current ranges used by farmers were generated in the past for crops yielding at much lower levels than today. Table 1 compares the SRs for maize and sugar beet at CK1. The presented ranges, in spite of elaboration in different regions of the world (Europe, USA), differ only slightly. This suggests their suitability for world-wide application. It is much more difficult to make a reliable assessment of the nutritional status of sugar beets or potato (Table 1). For example, the Bergmann' sufficiency ranges developed at BBCH 41 for sugar beet are not currently suitable for correcting the nutritional status of currently grown varieties. The last date of this crop fertilization with N must precede BBCH 33 [43,44].

Table 1. Sufficient ranges of key nutrient contents in crop plants at the first cardinal stage (CK1).

Nutrients (% or mg kg^{-1} of Dry Weight)	Maize		Sugar Beet	
	BBCH 17 Bergmann [28]	BBCH 17 Schulte and Kelling [45]	BBCH 41 Bergmann [28]	BBCH 33 Barłóg [43]
Nitrogen, N, %	3.5–5.0	4.0–5.0	4.5–5.5	3.8–6.0
Phosphorus, P, %	0.35–0.6	0.4–0.6	0.3–0.6	0.27–0.46
Potassium, K, %	3.5–4.5	3.0–5.0	3.8–7.0	3.8–8.6
Magnesium, Mg, %	0.25–0.50	0.3–0.6	0.25–0.8	0.12–0.45
Calcium, Ca, %	0.3–1.0	0.51–1.6	0.6–1.5	0.28–0.85
Zinc, Zn, mg kg^{-1}	30–70	25–60	20–80	15–45

Maize has been subjected to in-depth studies on its nutritional status at the onset of flowering (Table 2). The presented ranges, despite different origin in terms of geographical region and publication year, differ only slightly. The biggest differences concern the content of Ca and K. The main reason for these variations is the calibration of plant tests under conditions of significant differences in the content of soil Ca and K in the area of the conducted research.

Table 2. Evaluation of maize nutritional status based on nutrient sufficiency ranges for the early leaf—the beginning of flowering—CK2.

Nutrients (% or mg kg^{-1} of Dry Weight)	Authors			
	Schulte and Kelling [45]	Jones et al. [46]	Campbell and Plank [41]	Potarzycki [33]
Nitrogen, N, %	3.0–4.0	2.6–3.6	2.8–4.0	2.1–3.33
Phosphorus, P, %	0.3–0.45	0.22–0.4	0.25–0.5	0.23–0.35
Potassium, K, %	2.0–3.0	1.8–4.5	1.8–3.0	1.9–2.5
Magnesium, Mg, %	0.2–0.8	0.43–1.0	0.25–0.8	0.41–0.67
Calcium, Ca, %	0.2–1.0	0.27–0.34	0.15–0.6	0.28–0.36
Zinc, mg kg^{-1}	20–70	19–75	20–70	40–70 [1]

[1] corrected by author.

2.2. The Root System Architecture—RSA

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn. The uptake of nutrients related to the incorporation of ions or molecules into the plant's organism consists of a series of sequential processes that can be divided into three main ones:

1. Movement of nutrients along the soil/plant continuum:
    a. transport of ions/molecules from the soil solution towards the root surface,
    b. ingrowth of the root into soil patches rich in available nutrients;
2. Transport of nutrients adsorbed on the root surface through the plasma membrane into the cytoplasm;
3. Direct utilization of the nutrient in the root or its transport via the xylem to active plant tissues.

The processes mentioned in points 2 and 3 are extensively described in scientific books and extended reviews [16,47]. Here, we discuss the key processes related to root system growth during the growing season. The functions of the root system of crop plants can be considered from several points of view [48–50]:

1. Anchorage of the plant in the soil;
2. Water extraction from the soil to:

   a. stabilize the shoot temperature
   b. transport nutrients to the shoot;
3. Nutrient uptake from the soil solution;
4. Impact on rhizosphere processes through:
   a. release of organic compounds → a source of energy for microorganisms present in the rhizosphere
   b. release of protons or chelating agents → increase in nutrient availability
   c. deposition of carbon by dead roots → humus build-up;
5. Symbiotic associations with bacteria or fungi;
6. Storage organs, treated as main yield (sugar beets, cassava, sweet potato).

The root system, despite seasonal dynamics and spatial variability, is a conservative trait of the plant. It can be characterized as a three-dimensional structure, creating the root system architecture (RSA) [51,52]. The components that describe RSA include three main characteristics of the root system:

1. Primary root (PR) length, which determines the depth of a plant rooting;
2. Root branching patterns, which are represented by a number of characteristics, among others (i) number of lateral roots (LR), number of adventitious roots (AR), (ii) growth angle of LR and AR in relation to the primary root (s), (iii) root diameter, (iv) root length density (RLD);
3. Root hairs (RH), including length, diameter, number per root unit length or area.

Generally, on the basis of the plant branching patterns, the root systems of crops, that are botanically justified, are classified as taproots (dicotyledonous species, dicots) and fibrous roots (monocotyledonous species, monocots). The main components of the taproot system are PR, LS, and AR roots. The fibrous root system consists of PR, seminal roots, crown roots and AR roots [53].

The spatial distribution of roots in the soil profile is important for both the current rate of crop growth, as a decisive factor for the uptake of water and nutrients, and for maintaining soil fertility due to allocation of carbon. The spatial arrangement of the root system in the soil profile, in spite of its heterogeneity, can be described by specific parameters or indices. This concerns, first of all, the general shape of the root system profile down to the soil. The key parameters are: (i) distribution of the total root biomass, (ii) plant rooting depth, (iii) root length density [48,54]. Root distribution with depth can be best described using, for example, an exponential model by Gerwitz and Page [55]:

$$\mathrm{Y} = \mathrm{A}\left(1 - \mathrm{e}^{-\mathrm{cx}}\right) \tag{10}$$

where: Y—the cumulative fraction of roots between a soil layer of 0–10 cm and the depth x + 10 cm (cm); x—a defined soil layer below 0–10 cm; c—an empirical fitting parameter that determines the root distribution with depth.

This equation or others, more mathematically advanced, are used to define the effective rooting depth (ERD) as the key RSA parameter [56,57]. Most of the root biomass is present in the topsoil, decreasing exponentially with the soil depth. As estimated by Fan et al. [56] for main crops grown in a humid climate 50% of the root biomass is in the top 20 cm. The remainder part of roots, present in the subsoil, is important for water and nutrient uptake. Under conditions of drought, the uptake of water and nutrients from deeper soil layers is critical for both growth and yield maintenance [58]. The ERD is defined as the potential depth of the soil profile from which plant roots can extract the maximum amount of water available to plants from the soil during dry years. The soil layer, extending between the soil surface and the ERD, is known as the effective root zone (ERZ) [59]. This zone, depending on the assumption, covers 80% or even to 95% of the total root mass or root extent. As reported by Fan et al. [56], 50% of wheat root biomass is present within 16.8 cm of the soil surface layer, while 95% reaches down to 103.8 cm of the soil profile. In agricultural practice, the ERD is used to assess both the water resources for the currently grown crop and/or the dose of irrigation water. For example, the ERZ in the Czech Republic is estimated at 80–100 cm for winter cereals, and at 40–50 cm for potatoes [60].

The role of the subsoil in plant growth and yielding is usually ignored in the diagnosis of crop plant fertilization. This ERD is, in fact, used as a routine diagnostic tool to determine the content of mineral nitrogen (N_{min}). For most crops, this analysis is performed down to a depth of 90 cm [61]. Subsoil is an important storage of other nutrients, including P [62]. Current studies document that these P resources are used by plants, provided that the P balance in the topsoil is negative. This conclusion is probably the result of using powerful extractants to determine the available P [63]. A study by Barłóg et al. [64] clearly showed that extraction solution for N_{min} determination can also be used to determine the resources of other nutrients. As shown in Figure 9, the seasonal pattern of available P (0.01 M $CaCl_2$ extract; soil: solution ratio as 1:5), regardless of the season (crop), was stable. The P content was in a declining pattern in the soil profile. With the exception of 2005, its content was lower at crop maturity compared to spring. These P resources can be exploited by plants to up to 60% of its total content in the 0.9 m soil layer [65].

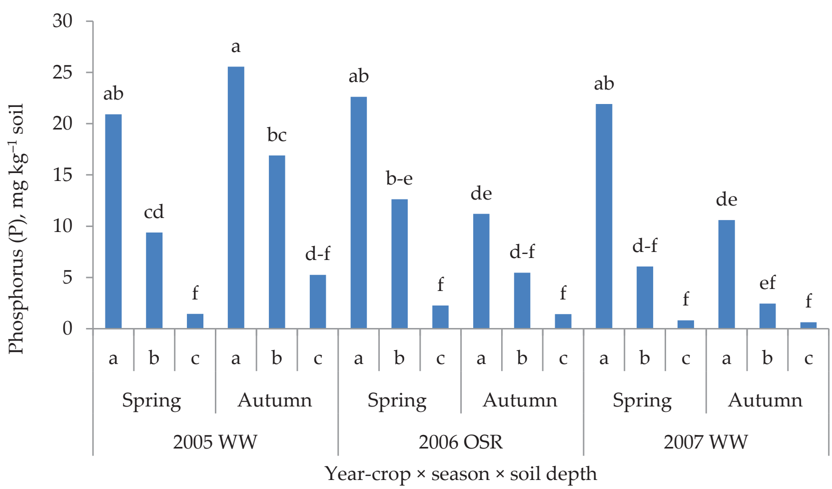

Figure 9. The seasonal patterns of available phosphorus distribution within soil layers (based on Łukowiak et al. [65]). Key: WW—winter wheat, OSR—oilseed rape. Letters indicate significant differences between treatments.

2.3. *Root System Growth during the Growing Season*

The genetically determined root system of plants is heterogeneous both in time and in soil space [48,66]. The first variable is inextricably linked with the plant's life cycle. Generally speaking, the growth of the root system as an integral part of the shoot system, is a result of both organs functional interdependences [67,68]. Maintaining a stable but temporary balance between the supply of water and nutrients to the shoot by the roots and the return supply of assimilates to the roots form the shoots is the basis that determines the plant growth rate, development of yield components, and yield [69].

The relationship between these two organs of the plant during its life cycle is not constant, as expressed by the ratio of the biomass of the shoot to the biomass of the root (S/R). Its value, as a rule, increases with plant growth (Figure 10). A frequently asked question concerns the relationship between shoot growth and the ability of the root system to supply the required amount of nitrogen [67]. In cereals, the highest rate of N uptake by roots occurs in the period from the end of tillering to the stage of full stem elongation (BBCH 29 to BBCH 37; Figure 6). For example, during this period, the rate of N uptake by winter rye plants on a plot fertilized with NPK and manure (long-term static experiment, existing 30 years before the study) was 3- and 10-fold faster compared to plants grown on a plot fertilized only with manure or on the absolute control [70]. Moreover, the rye root system on the NPK + manure plot was both shallower and more branched than on the absolute control [71]. This is in line with current studies on wheat, highlighting the importance of the early stages of stem elongation for the development of yield components [37]. Moreover, the period of the highest N uptake by winter rye confirms the well-defined CK1 (Figure 5).

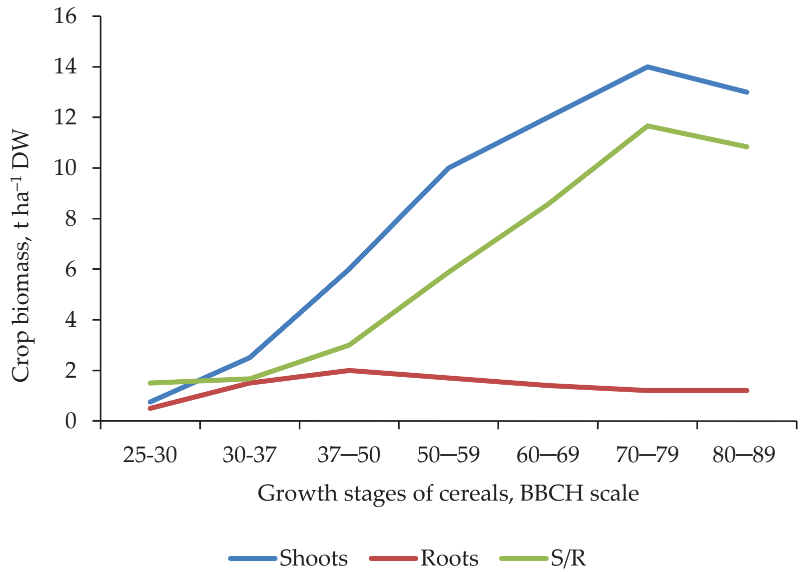

Figure 10. The general pattern of the growth of the root and shoot biomass of cereals during the growing season (based on Grzebisz [70]). Legend: R/S—root to shoot biomass ratio.

The second variable affecting the RSA concerns the impact of soil and environmental conditions on the development of the root system during the growing season. The primary factor of root growth is temperature, which determines the rate of all metabolic and physiological processes during a plant's life cycle [12]. The optimum temperature for root growth is much lower for plants from temperate than tropical climates [69]. The second factor is water, the function of which, similarly to temperature, cannot be separated into individual processes [72]. The third factor is soil fertility, which determines the efficiency of water and nitrogen [5]. The effect of soil fertility on RSA depends on the course of temperature and water conditions during the growing season. Any change in the environmental conditions for the worse (temporary water shortage, lower level of soil fertility, low availability of nitrate nitrogen) increases the plant's input into the root system size, mainly increasing its rooting depth—the primary root and root hair length, while reducing the development of lateral roots. The observed morphological changes are due to the actions of hormones, which are dependent on the availability of nitrate nitrogen [73,74].

The maximum demand for nutrients by a plant, as shown in Figures 4 and 5, occurs during the linear phase of the biomass accumulation by the crop. Soil inherent (quasi natural) resources of nutrients can be potentially high, but the plant's nutrient requirements at the maximum growth are higher than their supply to the plant from soil solution [8]. The rate of any given nutrient movement in the soil solution towards the root surface depends, among others, on the value of its diffusion coefficient. In pure water, the differences between diffusion coefficients for nutrients are small compared to their values in the soil solution (Table 3). The coefficients for NH_4^+ and K^+ are about 100-fold lower compared to the nitrate ion (NO_3^-). An even lower value is the attribute of the orthophosphate ion. Moreover, the differences between the values of the diffusion coefficients for all these ions increase with the decrease in the water content in the soil [75].

Table 3. Coefficients of effective diffusion for main nutrients in water and soil solution [1].

Nutrient	Ion	D_w, cm^2 s^{-1}	D_{eff}, cm^2 s^{-1}
Nitrogen	NH_4^+	1.96×10^{-5}	6.1×10^{-8}
	NO_3^-	1.90×10^{-5}	2.7×10^{-6}
Phosphorus	$H_2PO_4^-$	0.89×10^{-5}	$0.3–3.33 \times 10^{-9}$
Potassium	K^+	2.00×10^{-5}	$1–28 \times 10^{-8}$

[1] source: Raynaud and Leadley [76]; Clarkson [77].

The absorption of a given nutrient by the plant root results in a decrease in its concentration around the root. This phenomenon is called the depletion zone, which is specific for each individual nutrient [67]. The size of the nutrient depletion zone (NDZ) is determined by two key variables: (i) value of its diffusion coefficient (D_{eff}); (ii) soil exploitation time by the root (t). The influence of both variables on NDZ can presented in the formula:

$$NDZ = (2 \times D_{eff} \times t)^{1/2} \tag{11}$$

where: NDZ—the size of the depletion zone, cm; D_{eff}—diffusion coefficient of a particular nutrient, cm^2 s^{-1}; t—time, s.

The NDZ arises when:

(a) metabolic requirements of the above-ground parts of a plant are higher than the rate of nutrient supply to the plant from the soil solution;
(b) the effective diffusion of a nutrient is sufficiently high;
(c) the time of the plant root interaction with the soil in a particular soil zone is long enough.

The uptake of nutrients during the Yield Formation Period (YFP) of plant growth depends on the rooting depth and the root length density (RLD, cm cm^{-3}) of the growing crop. The effect of RLD on the size of NDZ is nutrient specific. The rate of nitrate nitrogen ion (NO_3-N) movement to the root is the most rapid of any nutrient, resulting in the fastest increase in NDZ around the root. Competition between neighboring roots occurs when their density exceeds 1–3 cm cm^{-3}. Maize with an RLD of 3 cm cm^{-3} absorbs about 70% of NO_3-N present in the soil solution. At the same time, the degree of P and K depletion does not exceed 5% and 10%, respectively [78]. Competition between the roots for P may occur, provided the RLD exceeds 30 cm cm^{-3} [79]. The RLD for crop plants rarely exceeds 2–5 cm cm^{-3}. The exception are grasses, for which this parameter is in the range of 3–20 cm cm^{-3} [79]. An apparent paradox for both traits of RSA is that the greatest RLD values in the topsoil, regardless of the crop, decline exponentially with depth [67]. Current studies on winter wheat and winter oilseed rape have shown that the critical RLD of 1 cm cm^{-3} was 32 and 45 cm, respectively [80]. These data indicate that there is no competition between roots for NO_3-N below this depth. However, it can be assumed that the presence of roots in the deeper soil layers has a significant impact on the yield. As recently presented by Grzebisz et al. [35], the content of NO_3-N in the soil layer (0.6–0.9 m) was the key nutritional factor that determined the yield of winter oilseed rape (WOSR). As shown in Figure 11, the greater the decrease in the NO_3-N content during YFP, the greater the WOSR yield obtained. During YFP, the sequential application of N_f creates rich N-NO_3 zones in the topsoil, while the deeper soil layers are, as a rule, much poorer in nitrate content. Nevertheless, no reduction in root growth is observed within this mega-phase, either in the topsoil or the subsoil [71,81]. The ingrowth of the primary root in the subsoil and the simultaneous growth of lateral roots in the rich NO_3-N niches in the topsoil can be explained by the *foraging strategy* of a crop [54,82]. This phenomenon entails the synchronization of both the local and systemic signals within a plant in response to the NO_3-N status in the soil profile. The decrease in concentration of NO_3-N in the subsoil, which is a typical phenomenon during YFP, leads to the increased flow of auxin to the apex of the primary root. As a consequence, it stops the growth of lateral roots within a soil zone poor in nitrates. At the same time, the induced systematic signal released by the apex of the primary root results in a compensatory growth of lateral roots in soil zones rich in nitrates [83]. The application of N_f by the farmer during the growing season leads to the formation of soil zones—*foraging patches* for a plant, which temporarily differ in the concentration of NO_3-N. Therefore, it can be concluded that a split N fertilization system is a useful way to increase the efficiency of the applied N_f.

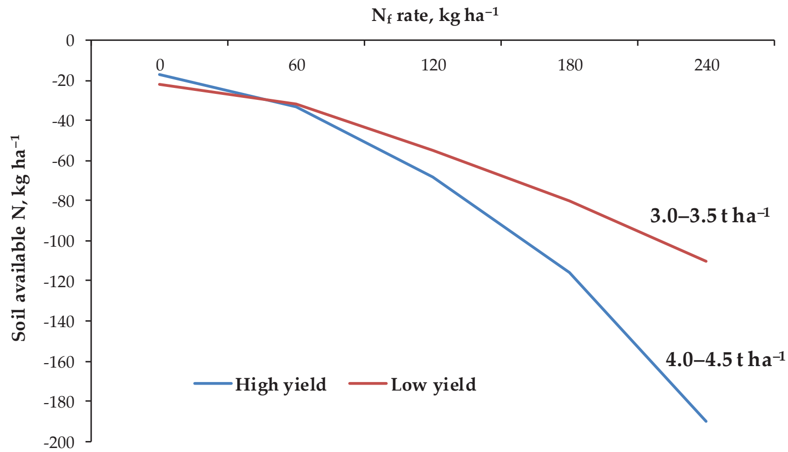

Figure 11. The amount of the soil and fertilizer N depleted during the Yield Formation Period (YFP) depending on nitrogen (N_f) rate. Key: High, Low yield of winter oilseed rape. (Based on Grzebisz et al. [35]).

3. Soil Factors Affecting FUE

3.1. Soil Texture

The most important soil physical properties include: soil texture, density, structure, porosity, consistence, temperature, air and color. Among them, soil texture is the basic physical feature that determines not only the other physical properties of the soil, but also the chemical ones [84]. The percentage and mineralogical composition of the smallest mineral fractions in the parent rock determines the primary soil potential to supply plants with nutrients, which is the function of weathering and transforming primary minerals [85]. In addition, the content of mineral colloids is positively correlated with soil organic matter (SOM), which in turn is a source of organic colloids, which have a great impact on the water retention of the soil, cation exchange capacity, erosion processes, as well as soil microbial activity [86]. SOM sequestration is achieved through various mechanisms which include the formation of clay-humic complexes, sorption of organic matter on clay particles, fixation of organic carbon in the crystal lattices of clays and the formation of organo-metallic compounds such as Ca, Fe and Al humates through humification processes [87,88]. In general, the greater the SOM concentration, the greater the sorption capacity of the soil, and potential for water retention in soil [89] and nutrients [90]. Numerous studies show that soils with a high proportion of clay particles have a higher content of nutrients than soils with a low content of nutrients, not only in terms of general forms, but also plant-available forms [91,92]. At the same time, the clay content affects the fixation and de-fixation processes of some nutrients, especially K^+ [91]. On the one hand, excessive fixation reduces the pool of mobile K^+ ions in the soil and reduces the use of potassium from fertilizers, especially in dry soil conditions. On the other hand, it prevents the leaching of potassium from the soil [93]. Moreover, adsorption and non-exchangeable ammonium nitrogen (NH_4^+) fixation in soil is highly dependent on clay mineral composition [94]. Another problem with soil texture is water infiltration and the leaching of nitrates (NO_3^-) resulting from ammonium nitrification. Coarser-textured soils are more susceptible to soil N loss following the leaching of NO_3^-, and thus have potentially lower FUE values [64]. Furthermore, soil texture largely affects fertilizer and soil P transformations in soils. In coarser-textured soils the content of labile P fractions after adding phosphorus fertilizers is higher than in clay and loam soils. Therefore, in these soils there is a high risk of P transfer from soil to water systems [95].

3.2. Water Content

One of the most important factors controlling nutrient uptake and utilization by plants is the water content of the soil. First of all, water determines the processes of nutrient release from the soil solid phase to the solution phase [96,97]. Water deficiency in soil negatively affects microbiological activity and the processes of mineralization/biological fixation [98]. Water is also essential for dissolving and releasing nutrients from mineral fertilizers, including controlled release fertilizer [99]. However, from the point of view of the process of uptake of nutrients by plants, two phenomena deserve special mention: mass flow and diffusion [100]. Water deficiency in the soil reduces the intensity of both processes, and thus leads to a reduction in the amount of nutrients flowing to the root surfaces [101]. In this aspect, the degree of plant reaction to water stress depends on the element and its function. According to Oliveira et al. [102], in maize the proportion of mass flow contribution to Ca, Mg, N, S and K transport was as follows: 100, 63, 56, 45 and 10%, respectively. This series clearly shows that the supply of plants with Ca and Mg may be severely limited in drought conditions, despite their relatively high concentration in the soil compared to other macronutrients [103]. Taking into account the diffusion processes, a water shortage in the soil will primarily limit the mobility of phosphate ions and micronutrients. Moreover, it will lead to the intensification of precipitation processes and the crystallization of amorphous compounds of phosphorus with other cations, depending on the pH [104]. As the water deficit in the soil increases, the proportion of pores filled with air increases, mechanical resistance increases, and the rate of root growth decreases. Under conditions of high soil oxygenation, the potential of the soil to supply plants with some micronutrients is reduced (Fe, Mn), whose higher oxidation state forms are less plant-available than the reduced forms [105]. The second group factors effecting NUE directly relates to the plant response (growth) and its ability to convert in biomass the assimilated/remobilized nutrients, especially nitrogen [106]. Water has a direct effect on root growth. In order to meet the demand for water, the roots constantly explore the soil, building a very complex, branched architecture [107]. An increase in the number of hairs and diameter root tips has been observed in plants under drought conditions. Root hairs greatly increase root-soil contact and the surface area available for adsorbing water and nutrients [108]. However, dense and deep root systems are not always good under all hydrological conditions, for example they poorly capture water from the topsoil under low rainfall conditions [109]. In drought conditions, the above-ground mass is reduced more than the underground mass, which in the case of a long-lasting drought may limit the inflow of assimilations and stop root growth, with all the negative effects of this phenomenon [110]. Lupini et al. [111] reported that water stress in durum wheat reduces the values of NUE, NUpE, and NUtE indices, regardless of the genotype. However, it should be remembered that excess water is just as harmful to plants as is its deficiency. One of the reasons for this is the reduction in the oxygen content in the soil needed for the respiration of plants and microorganisms [98]. In addition, large amounts of iron or manganese are released, which in excess may be toxic or interfere with the absorption of other nutrients. This phenomenon is particularly harmful in the cultivation of rice paddy on acidic soils [112].

3.3. Soil Compaction

Another important physical factor influencing nutrient uptake from soil, as well as their utilization from fertilizers, is soil compaction. Compaction affects plant growth by reducing the content of soil air and plant-available water, and the consequent restricted root growth results in the plant being unable to obtain an adequate amount of nutrients. Soil compaction can be assessed by measuring the following soil properties: bulk density, porosity and mechanical impedance [113]. Mechanical impedance is defined as a physical barrier to developing roots as a result of excessive bulk density. In general, root growth rates decrease sharply for soil mechanical impedance values between 0.8 and 3 MPa. On the other hand, when assessing soil compaction by soil bulk density, most authors give the value of 1.47–1.85 g cm^{-3} as critical for crops, depending on the percentage of clay [114,115]. The turgor in the cells in the elongation part of the roots determines their ability to overcome the mechanical resistance of the soil [116]. The greater it is, the greater the probability of root growth into the zone of compacted soil [117]. At the same time, root elongation is facilitated by root secretions and abraded side cells of the roots, which reduce the effect of the friction force [118]. When the mechanical resistance is too high, changes are observed at the physiological level (accumulation of solutes, reduction in the growth rate, new cell production) as well as anatomical (increase in the root diameter and the share of mechanical tissue in the direction of growth) [119,120]. The entire root system develops into less resistant parts of the soil, often forming a shallow system with the roots parallel to the soil surface [121]. According to Ramalingam et al. [122] the root length density at 30–60 cm soil depth decreased with hard compaction (to 70% of control) and increased with moderate compaction (to 135%). At the same time, the number of roots with a deep angle (i.e., 45° to 90° from the horizontal) correlated with the root length density and its proportion was lower in compacted soil. Considering the root architecture, the studies carried out so far have shown that deeper root growth is more important for N uptake than increased root density [123]. In this respect, it is necessary to remove the soil compaction in the subsoil. On arable land, the use of heavy machinery increases the risk of soil compaction especially in the subsoil [124]. Changes in the root architecture mean that the plant is unable to fully use nutrients, especially those whose main reservoirs are in deeper layers of soil [125]. Regardless of the soil depth, when the soil is characterized by excessive bulk density and/or mechanical impedance, the roots develop mainly in macro-pores [126]. This results in a poor supply of nutrients in plants under soil drought conditions, as the macro-pores in soil water retention only contribute to a small extent [98]. Another important issue with soil compaction is the loss of nitrogen from the soil through the emission of its gaseous forms into the atmosphere. As a result of soil compaction and the oxygen deficiency caused by this process, the activity of denitrifying bacteria increases and the production of N_2O and N_2 increases [127]. The emission of these gases to the atmosphere is favored by the low values of the parameters that define gas diffusivity in compacted soils [128]. According to Ruser et al. [129], high N_2O emissions in compacted soils occurred at a water-filled pore space > 70%. N_2 production took place only at the highest soil moisture level (>90% water-filled pore space) but it was considerably less than the N_2O-N emission in the most compacted areas in a potato field. Soil compaction also increases the volatilization of ammonia, as compared to uncompacted soils [130]. However, for this gas, the emissions are mainly determined by other soil physical and chemical characteristics [131].

3.4. Soil Temperature

Temperature has a substantial effect on some soil properties as well as root growth. Important processes depend on the temperature of the soil, such as: soil structure, aggregate stability, soil moisture content and aeration, soil pH, cation exchange capacity (CEC), soil microbial activities and organic matter decomposition [132]. A soil temperature between 2–38 °C increases the decomposition of organic matter by stimulating microbial activities and increasing the solubility of chemical compounds [133]. As a result of decomposition, the resources of N, P, S and other nutrients available to plants increase [134]. From the point of view of the nutritional status of plants, an extremely important temperature-dependent process is the availability of P to plants. Soils with low temperature have low availability of P because the release of P from organic material is limited [135]. Soil temperature also influences the P diffusion coefficient in the soil. Yilvainio and Pettovuori [136] observed that water-soluble P increased with soil temperature from 50 to 250 °C due to the increase in the movement of P in soil controlled by diffusion. Soil temperature also affects nutrient uptake by changing soil water viscosity and root nutrient transport. At low soil temperature, nutrient uptake by plants is reduced as a result of high soil water viscosity and low activity of root nutrient transport [137]. In general, low temperature decreases both root elongation and branching. However, low temperatures inhibit shoot growth more than root, leading to a high root/shoot dry matter ratio [138]. Vessel lignification can be delayed and axial hydraulic conductivity is higher in roots grown at low temperatures compared to high temperatures [139]. Thus, tomato, for example, showed that low soil temperature results in reduced root growth, tissue nutrient concentrations and, as a consequence, the amount of the component taken from the soil [140]. The unfavorable effect of higher temperature is marked in various ways. Too high a temperature may lower the CEC, and at the same time cause an increase in the concentration of hydrogen protons (increase in soil acidification) due to the high rate of soil organic matter decomposition. The plant's response to temperature changes depends not only on the plant species, but also on the content of nutrients in the soil. According to Xia et al. [141], negative effects of excessive temperature on P content and uptake occur especially in P-poor soils. The authors also found that an overly high root zone temperature reduced root vitality and plant phosphorus content, which in turn affected plant growth and light energy utilization efficiency.

3.5. Soil Reaction

Among a number of chemical parameters describing the chemical properties of soils, the use of nutrients from fertilizers is very much influenced by its pH [142]. This feature directly relates to the concentration of active H^+ protons in aqueous solutions, and indirectly it is a measure of the acidity or alkalinity of a soil. The influence of soil pH on the nutrient uptake of plants results from many different phenomena and processes. The most important ones include: effecting the content of plant-available forms of nutrients in soil; capacity and proportions between cations in CEC; activity of trace elements and heavy metals; soil microbial activity, biological N_2 fixation; emissions of ammonia and other gases from the soil [143,144]. Both too acidic and alkaline soils have a negative effect on nutrient uptake. However, the phenomena occurring in acidic and alkaline soils differ significantly in terms of processes contributing to their degradation. A significant problem of acidified soils is an increase in exchangeable aluminum (Al^{3+}) [145]. The content of this form of aluminum monomers rapidly increases in soils below pH 5.0–5.5 ([146]; Figure 12). An excessive amount of Al^{3+} ions in the soil negatively affects the nutrient uptake processes and plant growth [147]. Numerous studies show that even at the stage of nutrient uptake, unfavorable phenomena take place, such as the competition of Al^{3+} ions with other ions for attachment sites in the apoplast, in carriers, attachment to the ATPase of cytoplasmic membranes and disruptions in the operation of the proton pump [148,149]. An excessive content of Al^{3+} ions in the soil significantly reduces the uptake of Mg^{2+} ions. This is due to the similar size of the hydrated ions [150]. One of the most important consequences of the presence of exchangeable aluminum in the soil is the disturbance of the growth and development of the root cap, and consequently the shortening of the root length and unfavorable changes in its structure [151]. For most crops, even a small concentration of exchangeable aluminum (in nanomoles) in the root cells is a toxic factor for the metabolic, physiological, genetic and biochemical processes taking place in the plant [152]. The reduction in the root system negatively affects the use of nitrogen in fertilizers and increases the risk of nitrate being washed out from the soil [153]. Moreover, nitrate nitrogen, which is not taken up by plants, is reduced to gaseous compounds, including N_2O [154]. In highly acidic soils, apart from exchangeable aluminum, excessive amounts of manganese (Mn^{2+}) and iron (Fe^{2+}) can also appear, which can further disrupt the proper growth and development of plants [155].

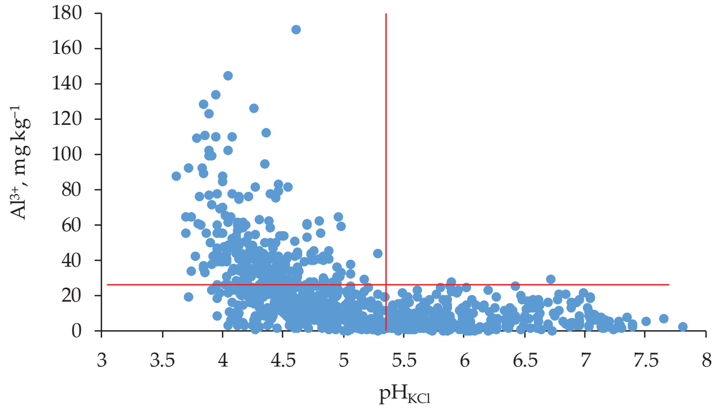

Figure 12. Exchangeable aluminum (Al^{3+}) content as a function of soil pH measured in suspension of 1 molar KCl (1:2.5, *w*/*v*). Sandy soils, western Poland (*n* = 986). The red lines indicate the critical points for soil pH and Al^{3+} content. Source: Błaszyk [146].

3.6. Soil Salinity

In arid or semi-arid climates, the problem is not soil acidification, but alkalization and salinity [156]. Under low rainfall conditions and a high evaporation rate, Na^+ ions, as well as various soluble salts, accumulate in the soil. Their excessive accumulation contributes to the significant advantage of OH^- ions over H^+ and, consequently, to an increase in soil pH to the level of 9–10 [157]. Soil alkalinity can also be increased by the addition of water containing dissolved bicarbonates, especially when irrigating with high-bicarbonate water [158]. The low osmotic potential of water in saline soils adversely affects water absorption by plants and nutrient uptake [159]. Salinity of soil significantly decreases P uptake by plants because phosphate ions precipitate with Ca ions contained in saline soils [160]. However, the alkalinity of soils is most often associated with the Na concentration [161]. Alkaline soils are characterized by unfavorable physical conditions, low content of plant-available forms of microelements and phosphorus, components determining nitrogen metabolism in the plant. During nutrient uptake processes, Na^+ ions compete for carriers with other nutrients in cationic form, in particular with K^+ ions [162]. This is a negative phenomenon because Na, dissimilar to K, negatively affects the activity of plant enzymes [163]. The reduced uptake of K^+ ions also means the insufficient or slower transport of NO_3^- from the roots to the above-ground parts, and thus poor efficiency of N from fertilizers [164]. Furthermore, an excess of Cl^- ions in the soil has a negative effect on NO_3^- uptake. However, as recent studies show, optimal NO_3^- vs. Cl^- ratios become a useful tool to increase crop yield and quality, agricultural sustainability and reduce the negative ecological impact of NO_3^- on the environment and on human health [165]. Under saline soil conditions, plants change their root architecture, which also has negative consequences for nutrient uptake [166].

3.7. Soil Organic Matter

The content of soil organic matter (SOC) in soil is one of the most important features influencing soil fertility [167]. Changes in SOC are associated mainly with changes in macronutrient contents, such as N, P and sulfur (S) which are chemically bound to carbon (C) in organic compounds [168]. Therefore, in systems where SOC content is declining, soil fertility declines over time and soils become increasingly dependent on the use of mineral fertilizers, especially nitrogen [169]. A total loss of organic N directly translates into a weaker potential of soils to release mineral forms that are taken up by plants. At the same time, under such conditions, the demand for N from fertilizers increases. Numerous experiences show that the most effective use of N from fertilizers is observed in the small dose range [9,170]. Conventional tillage with plowing can reduce SOC stocks by 30–60% [168]. Changes in NUE resulting indirectly from the increase in the degree of SOC degradation are confirmed by research of Luis et al. [171]. The authors calculated that over many years the efficiency of nitrogen fertilization application decreased from 68% in 1961 to 47% in 2010. This means that the use of N from fertilizers deteriorated and N losses to the environment increased by 21%.

In general, the transformation of native soil to agricultural uses leads to a decline in SOC levels [172]. However, agricultural land uses do not always result in losses of SOC. The rate and direction of changes in the C content in soils depend on the soil use system, irrigation, crops, and the level of organic matter return to the soil [173,174]. Failure to plow or use various simplified systems leads to the accumulation of SOC, especially in topsoil [175]. Reduced tillage in comparison with ploughing increased SOC stocks in the surface layer (0–10/15 cm) by 20.8% or 3.8 t ha^{-1}, depleted SOC stocks in the intermediate soil layers to 50 cm soil depth with a maximum depletion of 6.6% or 1.6 t ha^{-1} in 15/20–30 cm and increased SOC stocks in the deepest (70–100 cm) soil layer by 14.4% or 2.5 t ha^{-1} [176]. However, the use of natural and organic fertilizers is of greater practical importance in maintaining an appropriate SOC [177]. Szajdak et al. [178] reported that a yearly application of 30 t ha^{-1} of manure to light soil over 38 years doubled the SOC content. The increase in plant biomass as a result of the use of NPK fertilizers leads to an increase in the influx of C to the soil. Nevertheless, accumulation of C in soil is not favored by an excess of N in the soil from high fertilizer application rates and/or low plant uptake can cause an increase in the mineralization of organic carbon which, in turn, leads to an increased loss of C from soils [179].

3.8. Nutrient Shortage

Factors responsible for nutrient deficiency in crops can be divided into two main groups: (i) causing an absolute deficiency of nutrients in soil, resulting from low nutrient contents in the parent soil material, low level of SOC, nutrient losses from the soil, e.g., Mg leaching, long-term unbalanced crop fertilization practice neglecting nutrient depletion in soils through crop nutrient removal; (ii) causing an induced deficiency, resulting from factors that disturb the flow of nutrients to the root such as: improper moisture and temperature of soil, ion competition, factors responsible for root system size, etc. [12]. The natural source of most nutrients in the soil are primary and secondary minerals. As a result of weathering, often stimulated by the activity of living organisms, potentially available nutrients are released into the environment. Their reactions in soil and fate in the environment depend on the type of element. Some nutrients are strongly absorbed in the soil, others are easily lost (by leaching or emission). The first group includes K. The ions of this element can be absorbed in the soil in an exchangeable and non-exchangeable form [180]. The second type of adsorption prevents the elution of K^+ ions from soil, but on the other hand this leads to a reduction in the potential to supply plants with K. This phenomenon is responsible for the poor efficiency of K from fertilizers on soils rich in mineral colloids. The strength of the non-exchangeable K ion fixation increases in dry years, which further aggravates the symptoms of water stress [181]. Non-exchangeable adsorption may also apply to other cations, e.g., Mg. However, in relation to Mg, the degree of soil moisture has a greater practical importance, as this element is assimilated by plants as a result of a mechanism known as mass flow. Contrary to K, Mg is less readily absorbed [103]. This is one of the reasons for the relatively easy leaching of Mg from the soil. An absolute deficiency of K and Mg leads to a poor efficiency of N, as both elements greatly affect the metabolism and transport of N in plants [16]. Studies conducted on sugar beet show that Mg applied to the soil significantly increases the agronomic efficiency of N, but in the range of low doses of N (Figure 13). This indicates that an excess of nutrients in the soil may not lead to better FUE/NUE values. Phosphorus and micronutrient deficiencies in the soil are often the result of an inappropriate pH range in the soil. In an acidic reaction, the adsorption of P on iron and aluminum compounds increases, while in an alkaline pH, insoluble calcium phosphates precipitate in the soil [104]. An inappropriate soil pH also influences, directly or indirectly, the content of plant-available forms of K, Mg and Ca [144]. Thus, in order to restore the optimal conditions for the uptake of nutrients, it is necessary to regulate and/or constantly control the soil pH. If this does not help, then one option is to enrich the soil with nutrients to eliminate their absolute deficiency, or to support the plants by foliar fertilization.

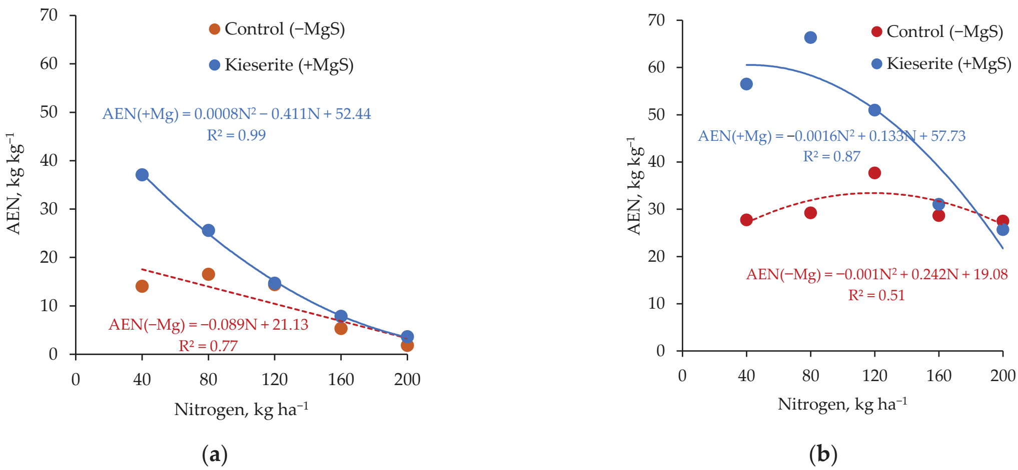

Figure 13. Effect of nitrogen application (40, 80, 120, 160 and 200 kg N ha^{-1}) on the agronomic efficiency of nitrogen (AEN), calculated for white sugar yield of sugar beet, depending on the availability of magnesium in the soil—kieserite application at a rate of 24 kg Mg ha^{-1}. Mean for two years for sandy soil (**a**) and loamy soil (**b**). Source: Pogłodziński et al. [182].

4. Innovations on the Fertilizer Market

Innovations in the fertilizer market involve two main areas of research activity. The first one concerns the process of obtaining raw materials and the production of fertilizers. The production of fertilizers, especially nitrogen ones, is energy-intensive and is a significant source of greenhouse gases. In this context, two strategies for the production of ammonia are considered: blue hydrogen—steam methane reforming with carbon capture and storage (CCS) and green hydrogen—electrolysis of water, to generate hydrogen and oxygen in a process driven by sustainable energy [183]. The second area of fertilizer production, mainly aimed at improving NUE indicators, concerns a number of application aspects and the chemical composition of fertilizers. Research shows that about 40–70% of N, 80–90% P and 50–70% of K from fertilizers is lost to the environment and cannot be used by plants, thus posing a threat to the environment [184]. For many years, the fertilizer industry has been improving and introducing Slow-Release Fertilizers (SRF) and Controlled-Release Fertilizers (CRF) [185].

The advantages of nitrogen fertilizers from the SRF and CRF groups derive from the following features: (i) they ensure a good supply of nitrogen to plants, especially in critical phases; (ii) they reduce the number of application rates; (iii) they reduce the nitrate content in plants; (iv) they limit nitrogen losses and reduce its negative impact on the environment [186,187]. With regards to nitrogen fertilizers from the SRF group, the delay of action is achieved by the formation of slightly soluble compounds, most often polymers based on urea and aldehydes, e.g., formaldehyde [188]. The condensation products of urea and other compounds can be used as solid (e.g., ureaform) or liquid fertilizers (e.g., urea-triazone). Research shows that liquid slow-release nitrogen fertilizer increases yields and nitrogen use efficiencies (NUE) in rape plants compared with a standard urea fertilizer [189]. For the production of CRF fertilizers, highly water-soluble compounds are used. Dissimilar to SRFs, controlled-release fertilizers (CRFs) are less influenced by soil temperature or texture, and they are not so dependent on soil microbiology [190]. The effect of delaying N release is achieved by covering the granules with a different type of protective layer (e.g., sulfur coatings, polystyrene, polyethylene, polyurethane, polysulfone resin and waxes coatings, siloxanes, etc.) [191–195]. The protective layers prevent the inflow of water from the soil to the inside of the granules and the dissolution of the contained compounds. The positive effects of different coated urea fertilizers on crop yield and NUE have been observed by many authors [196–198]. Recently, a great deal of attention has been paid to fertilizers using biochar and lignite for coatings, as they allow for the cheap production of CRF fertilizers [186]. Additionally, carbon-based materials, which contain humic acids act on plants such as biostimulants. The results of Wen et al. [199] also suggest that biochar-based slow-release nitrogen fertilizers could significantly improve the water-holding and water-retention capacity of soil. As a result, on the field scale, in rice cultivation, the optimal dose of N in the form of CRF (coated with lignosulfonates) fertilizer was 20% compared to using traditional nitrogen fertilizer [200]. In turn, according to Ghafoor et al. [198], biochar-based CRF fertilizers effectively reduce the nitrogen-release rate (69.8% of nitrogen was released after 30 days) and possess low nitrogen-leaching-loss amounts (10.3%), low nitrogen migrate-to-surface-loss amounts (7.4%), and high nitrogen-use efficiency (64.27%), as compared to other N fertilizers, consequently effectively promoting cotton plant growth. According to Guo et al. [201] fertilization of maize with CRF fertilizer with the addition of humic acids allows not only an increase in NUE, but also significantly reduces the emission of N_2O from the soil to the atmosphere by 29.1–32.6% compared to CRF fertilizer without humic acids. For the production of CRF fertilizers, nitrogen stabilizers are also used: urease and nitrification [185]. Among the various urease inhibitors, the most commonly used are N-(n-Butyl) thiophosphoric triamide (NBPT) and N-(n-propyl) thiophosphoric triamide (NPPT). The most commonly applied nitrification inhibitors are: 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin), dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP). The literature shows that the application of these inhibitors has considerably reduced inorganic N leaching, N_2, NO and N_2O emission while at the same time improving crop yield and N use efficiency [202,203] However, their effect on yield is very variable and depends on many factors. The application of fertilizer with urease inhibitors can increase the content of ammonium nitrogen in the soil by 10–59% compared to treatments without these inhibitors [204]. According to some researchers, it may increase the N resources in the soil and, consequently, gas losses of N (NH_3, N_2O) from the soil [205]. Therefore, the combined application of urease inhibitors with nitrification inhibitors reduces multiple losses associated with volatilization and denitrification [206]. Urea with urease and nitrification inhibitors can be used simultaneously to improve the N uptake, seed yield and grain protein contents, for example in quinoa [207]. Meta-analysis by Yang et al. [208]. showed that among the popular nitrification inhibitors, DCD was more effective than DMPP on increasing plant productivity. An increase in crop yield by DMPP was generally only observed in alkaline soil. This is confirmed by the results of Alonso-Ayuso et al. [209], who on soil with a pH of around 8.0 obtained after DMPP application allowed a 23% reduction in the fertilizer rate without decreasing maize yield and grain quality.

With respect to physical characteristics, in recent years urea has been produced with larger granules, facilitating mixing with fertilizers of similar grain size and bulk density, and allowing a wider spreading width compared to traditionally granulated urea. This is especially suitable for the fertilization of rice [210].

Innovations in the phosphorus fertilizer market also include the production of fertilizers with a controlled phosphorus release rate (CRFs). Their use increases the efficiency of using P from fertilizers (PUE) compared to traditional phosphorus fertilizers, and at the same time they reduce the negative impact of fertilization on the environment [191,211]. The rate of phosphorus release from fertilizers depends on a number of factors, including type and thickness of coating material, soil temperature and pH, humidity and microbial activity [193,212]. According to Fertahi et al. [213], 3 days after the application of phosphorus fertilizers, 100% P was released from water-soluble triple superphosphate (TSP) granular fertilizers, and only 60% from biopolymer coated TSPi. Next, Barbosa et al. [214] reported that biochar-based phosphate fertilizers have potential as a support material to increase the availability and efficiency of N use by plants. It should be mentioned that phosphorus fertilizers with a controlled phosphorus release rate also include: partially acidulated phosphoric (PAPR) and thermophosphates [215,216]. Stabilization of phosphorus transformations in the soil, and thus an increase in the potential P uptake from fertilizers, can be achieved by adding chemicals, the so-called phosphate boosters [217]. Their task is to decrease P-adsorption in soil and increase soluble-P from applied fertilizer-P [218]. Another solution for the future increase in PUE may be the addition of solubilizing bacteria [219]. In the foliar fertilizer market, fertilizers containing P in the form of phosphonates are now available. They have a beneficial effect not only on the nutritional status of plants, but also on their tolerance and resistance to fungal parasites [220].

The introduction of amino acids or other organic compounds of a biostimulating nature to the composition of fertilizers has also been a breakthrough in the foliar nutrition of plants. Amino acid molecules, distinct from technical salts or synthetic chelates, are electrically neutral, therefore the assimilation time of nutrients from fertilizers is short and their use will improve the nutrient use efficiency compared to traditional foliar fertilizers [221–223]. Glutamic acid has a particularly strong complexing effect [224]. On the other hand, some ammonium acids show a typical biostimulating character; for example, tryptophan, which is an auxin precursor. As demonstrated by Gondek et al. [225], NPKS soil fertilizer with the addition of thryptophan increased the maize biomass and the use of N and S from the fertilizer by 27% and 17%, respectively, compared to fertilizer without an amino acid. The incorporation of various organic and mineral substances into the soil together with fertilizers is an important way to improve the efficiency of using nutrients from fertilizers. As reported by Palanivell et al. [218], clinoptilolite zeolite application could contribute to an improved use of nitrogen, phosphorus, and potassium fertilizers to prevent soil, air, and water pollution. This treatment also improved nitrogen, phosphorus, and potassium use efficiency. The use of slow-release fertilizer hydrogels (SRFH) is also of interest. SRFHs are a combination of a super absorbent hydrogel (SAH) and a fertilizer with both water retention and slow-release properties [226]. Polymer super-absorbents are macromolecular compounds capable of absorbing water or physiological fluids in amounts much greater than their mass. They can be added to the soil or to fertilizers [227,228]. Among other things, chitosan-based hydrogels can be used as an additive to fertilizers [229].

The application of nanotechnology to the development of new types of fertilizers is considered to be one of the most promising options to significantly increase global plant production without negatively affecting the environment [230]. According to the European Commission [231], "Nanomaterial" means a natural, randomly generated or manufactured material containing particles in a free state or in the form of an aggregate or agglomerate in which at least 50% or more of the particles in the numerical particle size distribution have one or more dimensions in the range of 1 nm–100 nm. Nanoparticles are 100 to 1000 times larger than the size of the individual ions of nutrients that are involved in biochemical reactions [232]. However, they are in dimensions similar to or smaller than a number of anaotomous structures of plant tissues, e.g., plasmodesmata, cell wall pore sizes, or stomates [233]. Therefore, the presence of nanoparticles in foliar fertilizers improves the bioavailability of nutrients due to the nano-size, large specific surface area and greater reactivity of the compounds [234]. Fertilizers applied to the soil create the possibility that particles in the "nano" size may not be easily fixed between sheets of secondary minerals, and so not easily leached away from the soil [235]. The advantages of nanofertilizers also include the application of nutrients in a relatively smaller amount, ultimately reducing the cost of transport and at the same time improving the ease of application [236]. Nanofertilizers are usually divided into three groups: (i) classic fertilizer, but containing nano-scale particles; (ii) classic, traditional fertilizers with the addition of fertilizers in the form of nanoparticles; (iii) nanoscale coating fertilizer, referring to nutrients encapsulated by nanofilms or intercalated into nanoscale pores of a host material [237]. The nanocarriers used in the last group, such as zeolites, chitosan, clay and other nanomaterials, can provide plants with an even release of macronutrients during vegetation, which in the case of nitrogen and phosphorus improves their use in fertilizers [238,239]. Currently, the market of foliar fertilizers is developing intensively, which, apart from traditional compounds containing microelements, also contain noble metals, in particular silver ions, showing specific properties as pesticides on the "nano" scale [233,240]. Despite the large amount of literature on the potential use of nanofertilizers, there is little credible scientific evidence to demonstrate their advantage over traditional fertilizers. According to Kottegoda et al. [241] application of urea-coated hydroxyapatite nanohybrids (HA–urea) results in the enhancement of nitrogen use efficiency and reduces the environmental impacts of rice cultivation. Raguraj et al. [242] reported an increase in tea yield by 10–17%, while reducing the urea dose by 50% compared to traditional urea. Li et al. [243] reported that application of P in the formulation of nanoscale hydroxyapatite (nHA) had beneficial effects on soybean P and Ca content upon high precipitation intensities. However, the authors did not record any significant difference in the effect of fertilizers on the soybean biomass. A meta-analysis by Kah et al. [244] found that the median efficacy gain of nanofertilizers over conventional fertilizers was 19, 18 and 29% for categories of macronutrients, micronutrients and nanomaterials acting as carriers for macronutrients, respectively. However, Kopittke et al. [245] are critical of these results. The authors note that numerous researchers describe the positive aspects of nanofertilizers, but the experiments often lack an appropriate control object that would allow an objective assessment of their effects on plants. In terms of the potential use of nanofertilizers in the future, carbon nanotubes (e.g., consisting of 60 atoms of C-fullarens), which may contain nutrients, mainly microelements, or other bioactive compounds, are of interest [230,235,246]. As a result of such a formulation, future nanofertilizers will fully meet the criteria of CRF fertilizers.

5. FUE—A Message for Agricultural Practice

The stagnation in the increase in the crop yields is well-documented [247,248]. Despite considerable progress in breeding and the continual release of new varieties, the real improvement in NUE is small [249]. The challenge for the farmer to exploit the yield potential of the grown variety is:

1. Determine the maximum attainable yield (Y_{attmax}). This is the basis for choosing the most suitable variety for the actual climatic and soil conditions of the farm;
2. Identify soil conditions that constrain:
    a. growth and architecture of the root system
    b. water and nutrient availability;
3. Divide the whole field area into units of homogenous productivity;
4. Identify *Nitrogen Hotspots* both on the farm and on the specific field;
5. Observe the viability of plants at stages preceding the cardinal phases of yield formation;
6. Schedule the correction of the plant nutritional status during the season to exploit its yield potential.

The effective control of the set of factors indicated above is crucial to optimizing NUE. The general formula can be written as:

$$\text{NUE} = \frac{\text{Nitrogen Fertilizer Rate}}{\text{growth factors}} \tag{12}$$

The denominator includes all growth factors that determine the plant's uptake and utilization available N present in the soil-plant system during the growing season. The fractional value of all these factors, excluding N, should be $\leq$1.0 [5,8]. If the fractional value of a given growth factor approaches 1.0, its negative impact on NUE decreases and vice versa. The main challenge for the farmer in using N_f efficiently is to mitigate, or rather eliminate, the cause that leads to its fractional value drop below 1.0. Insufficient recognition of this value, and worse, the lack of action to control its value, is the main reason for low NUE both on the farm and worldwide [250,251].

The numerator of this equation is not the first but the second step in an effective control of the N_f use efficiency. The amount of N_f applied must meet at plant's requirement for N to exploit its yield potential, taking into account both the stage of growth and the spatial variability in plant N status [252]. The effective determination of the amount of N_f requires the use of appropriate diagnostic tools. The first dose of N_f, regardless of the crop, must be based on the content of N_{min} in the effective rooting depth of the currently cultivated plant [61]. A comprehensive view of the nutritional status of a plant in its full vegetation should be based on data on the content of both N_{min} and other nutrients in the soil [64]. The control of the plant nutritional status during the growing season, in fact, is limited to N. The chemometric diagnostic tools are good, but their use is limited. These methods are time-consuming and because of the delay between the sampling time and the delivery of the data to the farmer, they do not show the real condition of the plant N status. Real-time data can be obtained by using remote sensing techniques [253,254]. These methods rely on the absorption and reflection of solar radiation by a plant canopy. From this property of the plant, a number of crop characteristics can be determined in real time, such as (i) plant biomass, (ii) leaf area index, (iii) nitrogen content, (iv) chlorophyll content [255]. Biometric and nutritional data obtained at the cardinal stages of plant growth, combined with the required sum of the physiological effective temperatures at a given stage, form the basis for determining the crop growth rate. These data are used to forecast a plant's demand for N in strictly defined stages of its development. This is the basis for determining the appropriate dose of N_f. The spatial differences in the values of the field spectral indices can be used to develop a zonal map, showing the temporary crop N status. These maps are the basis for the application of N_f according to a plant's requirements in a well-defined field area [36,256].

6. Conclusions

The production efficiency of all nutrients, applied as mineral fertilizers, can be evaluated mainly through their impact on nitrogen use efficiency (NUE). The effectiveness of nitrogen present in the soil/plant system depends on the degree of correction of soil factors limiting plant growth and nitrogen uptake at critical stages of yield formation by the currently cultivated plant by other fertilizers, including lime. There are a number of soil factors that limit nitrogen uptake and reduce NUE indices. Some of them can be easily controlled by the farmer, for example soil compaction, pH, organic matter as well as content of plant-available nutrients that improve metabolism and the use of N by plants. Moreover, regardless of the crop, an N dose must be based on the soil content of N_{min} in the effective rooting depth and/or plant nutrition status at critical growth stages. Improvement of the parameters characterizing FUE/NUE parameters can also be achieved through the proper selection and use of innovative fertilizers. In recent years, slow- and controlled-release fertilizers produced with the use of biochar, lignite or other carbon-containing organic compounds have been of particular interest. In addition to the standard advantages of this type of fertilizer, a positive effect on the physical and chemical properties of the soil, as well as the growth of the root system can be achieved. Nanofertilizers are a new, promising direction of fertilizer development. Of particular interest is the possibility of using fullarens as nutrients carriers. Unfortunately, a reliable assessment of nanofertilizers is limited by a relatively small amount of data from field trials. Summing up, it is worth noting that regardless of the solution used to improve the NUE indicators, each action has a positive effect on the biogeochemical cycle of biogenic elements, and at the same time can help to protect the environment and reduce fertilization costs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11141855/s1, Table S1: A detailed analysis and evaluation of agronomic factors responsible for nitrogen gap (NG).

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Article

Band Phosphorus and Sulfur Fertilization as Drivers of Efficient Management of Nitrogen of Maize (*Zea mays* L.)

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Abstract: Increasing the efficiency of nitrogen use (NUE) from mineral fertilizers is one of the most important priorities of modern agriculture. The objectives of the present study were to assess the role of different nitrogen (N), phosphorus (P) and sulfur (S) rates on maize grain yield (GY), crop residue biomass, NUE indices, N concentration in plants during the growing season, N management indices and to select the most suitable set of NUE indicators. The following factors were tested: band application of di-ammonium phosphate and ammonium sulphate mixture (NPS fertilizer at rates 0, 8.7, 17.4, 26.2 kg ha^{-1} of P) and different total N rates (0, 60, 120, 180 kg ha^{-1} of N). In each year of the study, a clear trend of increased GY after NP(S) band application was observed. A particularly positive influence of that factor was confirmed at the lowest level of N fertilization. On average, the highest GY values were obtained for N2P3 and N3P1 treatments. The total N uptake and NUE indices also increased after the band application. In addition, a trend of improved N remobilization efficiency and the N contribution of remobilized N to grain as a result of band application of NP(S) was observed. Among various NUE indices, internal N utilization efficiency (IE) exhibited the strongest, yet negative, correlation with GY, whereas IE was a function of the N harvest index.

Keywords: agronomic efficiency; nitrogen gap; nitrogen remobilization efficiency; partial factor productivity; plant nutrient diagnosis; starter fertilization

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1. Introduction

Maize is one of the most important crops in the world. Among all cereals, it ranks second in terms of cultivated area (197 million hectares), just behind wheat (216 million hectares). Nevertheless, its global production between 2018 and 2020 was 1137 million tonnes, which was approximately 50% higher than the production of wheat [1]. According to the forecast, the global maize area in 2030 will expand even more (+5%), and the average yield, due to improving technology and cultivation practices, will increase by 10% [2]. In Poland, maize is also one of the dominant species. The area of maize grown for grain is about 1.0 million hectares, and maize for silage covers approximately 0.68 million hectares [3]. The yielding potentials of modern grain crop varieties are 11.6–12.8 t ha^{-1} [4]. In agricultural reality, however, they are lower (7.0–7.5 t ha^{-1}) and constitute about 60% of the breeding potential. There are many reasons for this state of affairs, one of which is inadequate agro-technology, a low level of fertilization and an imbalance of minerals.

The main factor determining photosynthesis, dry matter distribution and water efficiency is the concentration of soil available nitrogen (N_{min}) as well as the running N fertilization [5,6]. Unfortunately, the recovery of N from applied fertilizers (N_f) ranged from 30 to 50% only [7]. The part of N_f that is not consumed by the currently grown crop undergoes numerous processes that result in its loss to neighboring ecosystems, including both water and air [8,9]. Crop genetic improvements along with agronomic attempts to take control of N management in agriculture comprise a set of different strategies, which, in fact, focus on the increase in nitrogen use efficiency (NUE) [10–12].

At the beginning of plant growth, standard broadcast fertilization does not always ensure proper plant nutrition because, depending on soil properties, part of the component introduced into the soil in the form of fertilizer will land in places that are beyond the range of crop roots [13]. An alternative fertilization method is to place the fertilizer in close proximity to the seeds. This type of fertilizer application, also referred to as fertilizer placement, band application or initial, significantly accelerates the growth of maize and improves the plants' nutritional status at the beginning of the growing season [14]. This is due to both the increased concentration of minerals in close proximity of developing seedlings and the shaping of root architecture by N and P in particular [15–17]. In addition, this method of nitrogen application places the nutrient in a deeper, wetter soil layer, resulting in improved N uptake and limited N losses out to the environment [18]. Consequently, band placement not only prompts yield growth but also increases the values of NUE indices [17,19]. In general, fertilizer placement leads to an increase in maize yield in comparison to broadcast irrespective of the N fertilizer type [20]. Nevertheless, the use of ammonium phosphate proves to be the most productive of methods [21–23]. Despite numerous scientific findings on the beneficial impact of band application on maize yield and NUE, the N ratio still poses a problem and is little recognized, especially in conditions of high N demand. Apart from the many advantages of band fertilization, sprouting plants may become damaged, particularly when high rates of N fertilizers are used, where N comes in the form of NH_4-N [24].

Another problem associated with improving the use of N from fertilizers is the correct balance of nutrients. In temperate climate conditions, phosphorus (P) is of particular importance in maize cultivation. The initial growth of maize is slow; the root system is poorly developed, which causes low uptake of nutrients, including nitrogen, and at the same time, periodic temperature drops in the spring cause disruptions in phosphorus uptake and metabolism [13]. In addition, phosphate ion absorption and/or even the formation of insoluble compounds occur in soils, leading to poor use of phosphorus from broadcast fertilizers [25,26]. The risk of insufficient absorption of phosphorus in the spring by young maize plants can be mitigated by early application of the so-called starter fertilization [22,23]. Some authors recommend using this method on soils low in P and/or those with factors that hinder its uptake [27]. However, as research indicates, even in conditions where soil is rich in phosphorus, band application positively affects the grain yield of cereal crops [28]. Some authors also point out that on alkaline soils, the effect of ammonium phosphates on the maize dry matter and the use of P from fertilizers depend on their chemical composition, i.e., the N:P ratio [29]. Thus, a fertilizer containing diammonium phosphate (DAP) with additional sulfur (S) seems interesting and worth investigating. The results of this study suggest that early season growth of maize in some areas of the USA may benefit from S fertilizer application [30]. Most often, the assessment of the effect of ammonium phosphates on nitrogen management is carried out in the phase of physiological maturity of the plant. However, this assessment is an ex-post analysis and allows only for a determination of the sources of the component necessary during the period of growth and pouring of seeds/kernels.

A standard NUE assessment uses partial factor productivity (PNF), agronomic efficiency (AE) and apparent N recovery efficiency (RE) indices [31]. In the author's own study, a relatively new index was also tested, called the nitrogen gap (N_{gap}), in an attempt to determine its viability in the NUE assessment in specific field experiments [32]. The value of the N_{gap} indicates the pool of nitrogen not used in crop formation, resulting either from external (e.g., dry soil) or internal (e.g., imbalance of components) factors. After flowering, the role of soil nitrogen in grain yield formation diminishes, and the importance of N remobilized from green parts increases [5,33]. Therefore, in order to fully understand the impact of phosphorus fertilization on the uptake and use of N from fertilizers, it is also necessary to take into account the growth stages preceding the maturation of plants. The character of nitrogen management can be assessed by using indicators such as nitrogen remobilization efficiency (NRE) and contribution of remobilized N to grain N content (CNR) [34]. In this research, a hypothesis was formulated that the above-mentioned indicators remain in close relation with the grain yield and nitrogen accumulation in key points of maize growth, i.e., in the sixth–seventh leaf stage and at the beginning of flowering. Furthermore, the research hypothesis states that band application of DAP with added S improves NUE and prompts the reduction in the total N rate. This assumption was based on the following facts: low mobility of P in acidic soil, the specific demand of maize for P in the initial phase of growth and the stimulating effect of S on the metabolism of N compounds [22].

The objectives of the present study were: (i) to determine the maize yield-forming reaction to increasing rates of nitrogen fertilization in comparison to various rates of band application of NP(S); (ii) to evaluate the effect of NP(S) fertilization on the nutritional condition of maize at the seventh leaf stage, beginning of flowering and technological maturity; (iii) to establish the correlation between the level of maize yield, NUE indices and nutrient content with respect to the interaction of N and P(S) fertilizations.

2. Results

2.1. Grain Yield and Crop Residue Biomass

The average grain yield (GY) in both years (2019–2020) was at a similar level of 8.45 and 8.00 t ha^{-1}, respectively. The difference was 5.6%. The higher grain yield in the first year resulted from better weather and soil conditions at the start of the growing season—a damp spring and a high concentration of mineral nitrogen in soil. Unfortunately, in 2019, during intensive maize growth, kernel silking and at the beginning of kernel pouring, in June and July, weather conditions were unfavorable for the formation of the basic elements of the crop structure (Supplementary Materials, Table S1). Later precipitation only increased the dry matter of the vegetative parts, whereas in 2020, summer precipitation did not limit the silking and pouring of kernels. Consequently, the yield of post-harvest residue in 2019 (10.2 t ha^{-1}) was considerably higher (by 34.2%) than in 2020 ($F_{1,78}$ = 44.9; p < 0.001).

In the given years, no significant statistical differences between the fertilization treatments were confirmed. However, in each year of the study, a clear and unequivocal trend of increased GY after NP(S) band application was observed (Supplementary Materials, Figure S1). A particularly positive impact of this type of N application was confirmed at the lowest level of nitrogen fertilization (N1). At that level, the greatest GY was obtained after an application of the highest rate of NPS (N1P3 treatment). In 2019, the difference in relation to the absolute control (N0P0) was 22.5%, and in 2020, 24.3%. At N2, high increases in GY were also observed after the application of the highest NP(S) rate (N2P3). For N3, the differences in the years were transparent. In 2019, higher rates of ammonium phosphate lowered GY. In 2020, the highest increase in GY was obtained for the N3P3 treatment. In relation to the control, the difference was 31.8%. For comparison reasons, the maximum growth of GY in the treatment without NPS, but with the highest rate of total N, was only 16.4%.

The analysis of variance showed a significant impact of the fertilizing factor on the mean values of GY for the two years (Figure 1a). Significant differences were recorded between the control (N0P0) and N2P3 and N3P1. Regardless of the N rates (without taking N0P0 into consideration), an average influence of the ammonium phosphate and ammonium sulphate mixture on GY was as follows: P0 (7.82) < P1 (8.10) < P2 (8.54) < P3 (8.81 t ha^{-1}). Meanwhile, the impact of total N rates on GY was as follows: N1 (8.00) < N2 (8.33) < N3 (8.62 t ha^{-1}). In the above series, GY growth as a result of the maximum NP(S) application rate was 12.7%, and the total nitrogen rate was 7.8%.

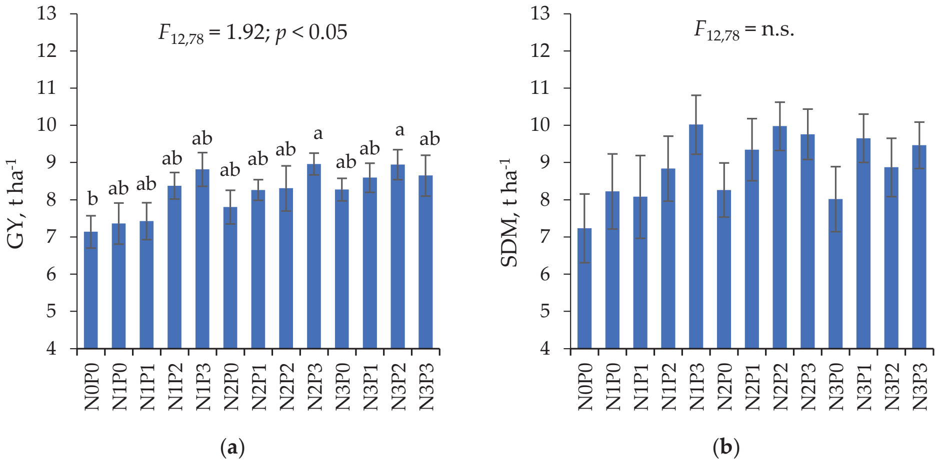

Figure 1. Maize grain yield, GY (**a**) and crop residues biomass, SDM (**b**) depending on fertilization treatments. Two-year average values. Means within a column followed by the same letter indicate a lack of significant difference between the fertilized treatments (HSD test). Hatched bars represent 2 × SEM ranges.

The studied factor did not differentiate the dry matter of post-harvest residue—straw yield (SDM)—in any significant way. However, fertilization clearly increased the dry matter of the vegetative parts. In both years, AP application had a positive effect on SDM, especially in N1 and N2 treatments. A difference between the years was registered for the N3 treatment. In the first year (2019), the higher rate of AP did not stimulate SDM at the same level as in 2020 (Figure S1).

On average, over the two years of study, the highest increase in SDM was obtained for N1P3, N2P2 and N2P3 treatments (Figure 1b). The difference in SDM between the nitrogen control (N0 = 7.23 t ha^{-1}) and the highest rate of total nitrogen (N3 = 9.00 t ha^{-1}) was approximately 24.5%, while the difference between the phosphorus control (P0 = 7.93 t ha^{-1}) and P3 treatment (9.22 t ha^{-1}) was approximately 16.3%. Despite this, these differences were not significant.

The biological maize yield (the sum of GY and SDM) also increased as the rates of N and P became higher, regardless of the year (Figure S1). In 2019, the highest biological yield was obtained under the N1P3 treatment, and in 2020, under N3P3.

The studied factor did not exert any major impact on the harvest index (HI). As opposed to GY and SDM, no clear trend was observed in terms of the general effect of fertilization. The parameter for N0P0 was 46.9% and 54.6%, depending on the year, whereas the maize fertilized with N and P showed HI values ranging from 41.0% (N2P2) to 49.5% (N3P2) in the first year, and from 49.7% (N2P2) to 54.7% (N3P0) in the following year.

2.2. Nitrogen Concentration and Accumulation at Maturity

The nitrogen content in maize significantly depended on the growing season, fertilization treatment and the studied part of the plant (Table 1). In the first year (2019), the N content in grain and in straw was higher than in 2020. At the same time, the accumulation of total N was also considerably higher in 2019 (269.9 kg ha^{-1}) than in 2020 (216.9 kg N ha^{-1}). The nitrogen harvest index (NHI) was, in turn, lower in 2019 (55.2%) than in 2020 (60.9%).

The studied factor notably influenced the content of N in grain (Ng) and in crop residues (Ns) in each year (Table S2). On average, for the two years of study, the greatest content of N in grain was registered for the treatment with the maximum fertilization rate of N and P (N3P3). The use of band application of DAP caused a particular increase in Ng at the level of N1. A similar interrelation was also obtained for Ns (Table 2). As a result of the fertilization impact on N concentration in grain and in crop residues, as well as on dry matter of the earlier mentioned maize parts, real differences in the accumulation of total N (TN) were recorded. A significant increase in TN in comparison to the control (N0P0) was registered in the following treatments: N1P3, N2P2, N2P3, N3P2 and N3P3. Depending on the treatment, the difference was 51.0–56.8%.

Table 1. Nitrogen (N) concentration and accumulation in maize at maturity (harvest) depending on fertilization treatments.

Year Treatment	N in Grain $g\ kg^{-1}$	N in Crop Residues $g\ kg^{-1}$	N in Grain $kg\ ha^{-1}$	N in Crop Residues $kg\ ha^{-1}$	Total N $kg\ ha^{-1}$	N Harvest Index %
Effect of the year (Y)						
2019	17.5 ± 0.3 ^a^	11.9 ± 0.3	148.2 ± 4.0 ^a^	121.7 ± 4.2 ^a^	269.9 ± 7.4 ^a^	55.2 ± 0.7 ^a^
2020	16.4 ± 0.3 ^b^	11.2 ± 0.2	131.5 ± 3.4 ^b^	85.3 ± 3.0 ^b^	216.9 ± 5.9 ^b^	60.9 ± 0.6 ^b^
Effect of the treatment (T)						
N0P0	14.3 ± 0.5 ^b^	10.3 ± 0.2	102.1 ± 6.7 ^d^	73.9 ± 9.1 ^b^	176.0 ± 15.3 ^b^	58.8 ± 1.6
N1P0	15.5 ± 0.8 ^ab^	11.1 ± 0.6	114.6 ± 9.5 ^cd^	92.1 ± 16.5 ^ab^	206.6 ± 25.6 ^ab^	55.4 ± 2.0
N1P1	16.4 ± 0.5 ^ab^	11.6 ± 0.8	122.1 ± 8.6 ^bcd^	95.6 ± 14.7 ^ab^	217.6 ± 21.8 ^ab^	56.1 ± 2.8
N1P2	16.8 ± 0.6 ^ab^	10.4 ± 0.7	141.4 ± 7.3 ^abc^	90.3 ± 7.3 ^ab^	231.6 ± 13.5 ^ab^	61.0 ± 1.4
N1P3	17.4 ± 0.4 ^ab^	12.0 ± 0.7	153.4 ± 8.3 ^ab^	121.3 ± 11.6 ^a^	274.7 ± 18.5 ^a^	55.8 ± 1.6
N2P0	16.7 ± 1.3 ^ab^	12.6 ± 0.1	130.9 ± 9.5 ^abc^	104.3 ± 9.6 ^ab^	235.2 ± 18.3 ^ab^	55.6 ± 1.6
N2P1	17.0 ± 0.7 ^ab^	10.5 ± 0.5	140.3 ± 5.4 ^abc^	99.0 ± 9.9 ^ab^	239.3 ± 14.6 ^ab^	58.6 ± 1.6
N2P2	17.7 ± 0.7 ^ab^	11.8 ± 0.6	146.9 ± 11.1^abc^	118.8 ± 9.8 ^a^	265.7 ± 18.1 ^a^	55.3 ± 2.3
N2P3	16.7 ± 0.7 ^ab^	12.2 ± 0.5	149.8 ± 5.1 ^abc^	119.8 ± 9.1 ^a^	269.6 ± 13.7 ^a^	55.6 ± 1.3
N3P0	17.6 ± 0.6 ^ab^	11.9 ± 0.9	145.9 ± 6.5 ^abc^	96.8 ± 12.6 ^ab^	242.7 ± 18.8 ^ab^	60.1 ± 2.1
N3P1	17.6 ± 1.1 ^ab^	10.8 ± 0.5	151.4 ± 8.9 ^abc^	103.1 ± 5.8 ^ab^	254.6 ± 13.8 ^a^	59.5 ± 0.9
N3P2	17.8 ± 0.3 ^ab^	13.0 ± 0.4	159.0 ± 7.4 ^ab^	115.4 ± 10.5 ^ab^	274.3 ± 13.9 ^a^	57.9 ± 2.4
N3P3	18.5 ± 0.2 ^a^	12.1 ± 0.6	160.5 ± 10.3 ^a^	115.5 ± 9.0 ^ab^	276.0 ± 15.7 ^a^	58.2 ± 2.1
ANOVA results (significance level)						
Year	*	n.s.	***	***	***	***
Treatment	*	n.s.	***	**	***	n.s.
Y × T	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

***, **, * significant at $p < 0.001$, $p < 0.01$, $p < 0.05$, respectively; n.s.—non-significant; means within a column followed by the same letter indicate a lack of significant difference between the fertilized treatments (HSD test).

The average impact of P(S) rates on TN over the two years was as follows: P0 (228.2) < P1 (237.2) < P2 (257.2) < P3 (274.3 kg ha^{-1}). For comparison reasons, the effect of the N rates was as follows: N1 (8.00) < N2 (8.33) < N3 (8.62 t ha^{-1}). The maximum difference for NPS rates was 20.2%, and for the full rate of N, it was 7.8%. The NHI values were not significantly differentiated by the application of NPS fertilization. The study did not prove any notable influence of ″year × NP(S) treatments″ interaction on the content or accumulation of nitrogen.

2.3. Nitrogen Use Efficiency Indices

The values for nitrogen fertilization efficiency indices are shown in Table 2. It is clear that the NP(S) treatments had a significant effect on indices such as PFP, PNB, RE, IE and N_{gap}. For comparison purposes, the growing season considerably influenced such indices as PNB, IE and N_{gap}. The studies did not confirm any great impact of ″year × NP(S) treatments″ on the values of the above-mentioned indices, however. In relation to PFP and N_{gap}, it was the total N fertilization that played the vital role. Higher N rates lowered PFP, yet increased N_{gap}. NP(S) fertilization brought the values of PFP up, regardless of the N fertilization levels (except for the N3P3 treatment). In relation to N_{gap}, a reverse interdependence was obtained. Along with the P increase, the N_{gap} value became lower.

Table 2. Nitrogen use efficiency (NUE) indices depending on fertilization treatment.

Year Treatment	PFP kg kg^{-1}	AE kg kg^{-1}	PNB kg kg^{-1}	RE kg kg^{-1}	IE kg kg^{-1}	PE kg kg^{-1}	Ngap kg N ha^{-1}
Effect of the year (Y)							
2019	86.3 ± 6.1	10.3 ± 3.4	1.51 ± 0.10 ^{a}	0.81 ± 0.14	28.6 ± 0.39 ^{b}	13.4 ± 1.8	53.2 ± 7.2 ^{a}
2020	80.8 ± 5.3	11.3 ± 2.1	1.32 ± 0.08 ^{b}	0.69 ± 0.07	35.5 ± 0.43 ^{a}	18.4 ± 1.0	48.2 ± 6.8 ^{b}
Effect of the treatment (T)							
N0P0	–	–	–	–	41.3 ± 1.5 ^{a}	21.3 ± 22.4	–
N1P0	122.8 ± 9.2 ^{a}	3.8 ± 13.3	1.91 ± 0.16 ^{c}	0.51 ± 0.51 ^{ab}	36.9 ± 1.7 ^{b}	−108.6 ± 146.8	−1.4 ± 4.5 ^{c}
N1P1	123.8 ± 8.6 ^{a}	4.8 ± 7.8	2.03 ± 0.14 ^{bc}	0.69 ± 0.33 ^{ab}	35.2 ± 2.0 ^{bc}	28.3 ± 7.5	−1.8 ± 4.0 ^{c}
N1P2	139.6 ± 6.0 ^{a}	20.6 ± 5.7	2.36 ± 0.12 ^{ab}	0.93 ± 0.17 ^{ab}	36.5 ± 1.1 ^{ab}	20.3 ± 2.8	−9.6 ± 2.3 ^{c}
N1P3	146.9 ± 7.5 ^{a}	27.9 ± 10.5	2.56 ± 0.14 ^{a}	1.64 ± 0.30 ^{a}	32.4 ± 1.2 ^{bc}	10.1 ± 6.8	−13.4 ± 3.8 ^{c}
N2P0	65.0 ± 3.8 ^{bc}	5.6 ± 5.8	1.09 ± 0.08 ^{de}	0.49 ± 0.16 ^{ab}	33.8 ± 1.8 ^{bc}	−197.5 ± 131.6	55.0 ± 3.6 ^{b}
N2P1	68.9 ± 2.3 ^{bc}	9.4 ± 5.3	1.17 ± 0.04 ^{de}	0.53 ± 0.16 ^{ab}	35.0 ± 1.3 ^{bc}	15.2 ± 7.6	51.0 ± 2.7 ^{b}
N2P2	69.2 ± 5.1 ^{bc}	9.8 ± 7.4	1.22 ± 0.09 ^{de}	0.75 ± 0.18 ^{ab}	31.4 ± 1.7 ^{c}	−75.5 ± 91.0	50.6 ± 5.2 ^{b}
N2P3	74.7 ± 2.4 ^{b}	15.2 ± 4.1	1.25 ± 0.04 ^{d}	0.78 ± 0.14 ^{a}	33.5 ± 0.8 ^{bc}	16.9 ± 3.6	45.4 ± 2.2 ^{b}
N3P0	46.0 ± 1.7 ^{c}	6.3 ± 3.0	0.81 ± 0.04 ^{e}	0.37 ± 0.12 ^{b}	34.9 ± 1.6 ^{bc}	16.7 ± 5.8	111.1 ± 2.4 ^{a}
N3P1	47.7 ± 2.2 ^{c}	8.1 ± 3.4	0.84 ± 0.05 ^{de}	0.44 ± 0.11 ^{b}	33.9 ± 0.9 ^{bc}	4.5 ± 11.1	108.4 ± 3.1 ^{a}
N3P2	49.7 ± 2.2 ^{bc}	10.0 ± 3.4	0.88 ± 0.04 ^{de}	0.55 ± 0.12 ^{ab}	32.9 ± 1.4 ^{bc}	11.7 ± 8.0	105.4 ± 3.4 ^{a}
N3P3	48.1 ± 3.0 ^{c}	8.4 ± 3.9	0.89 ± 0.06 ^{de}	0.56 ± 0.12 ^{ab}	31.4 ± 1.1 ^{c}	−10.0 ± 23.6	107.6 ± 5.1 ^{a}
ANOVA results (significance level)							
Year	n.s.	n.s.	***	n.s.	***	n.s.	*
Treatment	***	n.s.	***	*	***	***	***
Y × T	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

***, * significant at $p < 0.001$, $p < 0.05$, respectively; n.s.—non-significant; means within a column followed by the same letter indicate a lack of significant difference between the fertilized treatments (HSD test). PFP—partial factor productivity of N; AE—agronomic efficiency of N; PNB—partial N balance; RE—apparent N recovery efficiency; IE—internal N utilization efficiency; N_{gap}—N gap.

Negative N_{gap} values for N1 prove that N deficiency ensures a maximum yield and, at the same time, potential N soil mining, whereas for N3 and N2, the positive index values confirm the surplus of N that was not transformed into GY. The agronomic efficiency of N (AE) rose as the P rate increased. The highest AE value was obtained for the N1P3 treatment. Moreover, PNB and RE values were the highest for N1P3. The lowest values of both indices were obtained for N3, without a concomitant use of NPS. At the same time, the highest IE value was recorded in the control, while the lowest values were obtained for N2P2 and N3P3 treatments. Out of all the N and P fertilization treatments, the best use of TN was obtained for N1P0 and N1P3. Physiological N efficiency (PE) varied significantly among treatments. It is, however, worth mentioning that the lowest values were registered for the treatments without phosphorus (N1P0 and N2P0). Principal component analysis (PCA) was used to determine the relationships between the GY and NUE indices. The correlation matrix can be found in the Supplementary Materials (Table S3). The results of the PCA procedure were visualized in biplots (Figure 2). In addition, the interdependencies of the properties were analyzed with Pearson correlation coefficients (Table S4).

Based on the PCA analysis, three main components, representing the GY, SDM, N content and NUE indices, accounted for 89.9% of the total variance. The first principal component (PC1) explained 39.4% of the total variability, and the next two components (PC2 and PC3), respectively, 34.6% and 18.8% of the total variance (Table S3). As the two first principal components dominated, the results of the PCA analysis are presented on the PC1–PC2 biplot. PC1 consisted of variables related to the SDM, TN, RE and IE, as well as the GY and NHI. The loading exerted by PFP, N_{gap} and PNB influenced PC2. As shown in Figure 2, the GY was significantly related to the TN, IE, NHI and SDM. However, the GY showed negative relationships with the IE and NHI. This resulted from the fact that, in 2019, maize developed a greater biomass of crop residues than in 2020, thus lowering the share of N accumulated in grain (NHI) and N utilization efficiency (IE). The second group of parameters consists of such indices as RE, AE, PE, PNB, PFP and N_{gap}. A particularly strong correlation was noted between PFP and N_{gap} parameters. The year and fertilization treatments modified the values of the investigated parameters, as demonstrated by both PCA biplots. On the PC1–PC2 biplot axes, most of the treatments were grouped closest to the Tukey median (in the bagplot) or in the bagplot cover region. Only two treatments (N3P0 in 2020 and N1P3 in 2019) were separated by a significant distance from the Tukey median. At the same time, both variants were on the opposite side of the axis representing such indices as SDM, RE and NHI. On the axis representing NUE indices on the opposite sides of the median were variants from the N3 group and variants from the N1 group but with phosphorus at the rates of P2 and P3, while on the axis representing GY, on its opposite side, treatments such as N1P0, N1P1 (2020) and N2P2, N3P3 (2019) were placed. Close to the axis but near the Tukey median, treatments such as N2P2 and N3P3 from 2020 were found.

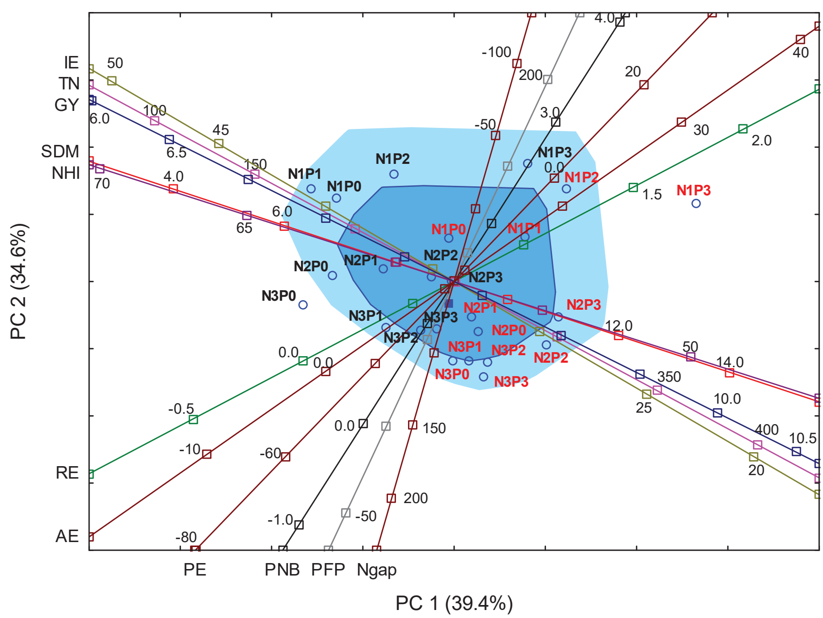

Figure 2. Principal component analysis (PCA) biplot of the maize yield and N use efficiency indices. The dark blue square denotes the Tukey median, the blue square is the bagplot, the light blue square is the bagplot cover. Key: GY—grain yield; SDM—crop residues (straw) biomass; TN—total N accumulation; NHI—N harvest index; PFP—partial factor productivity of N; AE—agronomic efficiency of N; PNB—partial N balance; RE—apparent N recovery efficiency; IE—internal N utilization efficiency; N_{gap}—N gap. The treatments in 2019 are marked in red and the treatments in 2020 in black.

2.4. Nitrogen Status of Maize

The nutritional assessment was carried out in the seventh leaf stage and at the beginning of flowering. The growing season had a significant impact on the maize dry matter in the first term, as well as on its N content (Table 3). No significant interaction was found for "year × fertilization treatments". Greater dry matter (DM) was obtained in the first year, however, with a slightly lower N content. As a result, N accumulation in plants in 2019 was higher than in 2020.

Table 3. Effect of the growing season and fertilization treatments on maize dry matter (DM) and nitrogen (N) status at the seventh leaf stage and at the beginning of flowering.

Year Treatment	Seventh Leaf Stage			Beginning of Flowering Stage		
	DM g Plant^{-1}	N Content g kg DM^{-1}	N Content g Plant^{-1}	DM g Plant^{-1}	N Content g kg DM^{-1}	N Content g plant^{-1}
			Effect of the year (Y)			
2019	9.57 ± 0.31 ^{a}	40.0 ± 0.5 ^{b}	0.39 ± 0.01 ^{a}	89.0 ± 2.3 ^{b}	26.1 ± 0.5 ^{a}	2.33 ± 0.07 ^{a}
2020	7.18 ± 0.23 ^{b}	42.1 ± 0.7 ^{a}	0.30 ± 0.01 ^{b}	114.5 ± 2.9 ^{a}	18.0 ± 0.4 ^{b}	2.08 ± 0.06 ^{b}
			ANOVA results (significance level)			
Year	***	*	***	***	***	**
Treatment	n.s.	n.s.	n.s.	n.s.	n.s.	***
Y × T	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

***, **, * significant at $p < 0.001$, $p < 0.01$, $p < 0.05$, respectively; n.s.—non-significant; means within a column followed by the same letter indicate a lack of significant difference between the fertilized treatments (HSD test).

In general, NP(S) application improved maize nutritional status in terms of N content. However, these changes were not statistically significant. The differences in comparison to the control were particularly visible at N1, while for N3, the differences in N content were inconclusive (Table S2).

As opposed to the first term, at the beginning of flowering, fertilization significantly differentiated N accumulation in plants (Figure 3). Both N and P fertilization increased the content of N in plants. For N1, along with the NP(S) rate increase, N accumulation rose by 13.3%. For N2, the maximum accumulation of N was recorded for treatments with P1 and P2 rates, while for N3, the highest N accumulation was registered after an application of the highest NPS rate, i.e., P3. The differences between N accumulation for P0 and other treatments were approx. 12–13%.

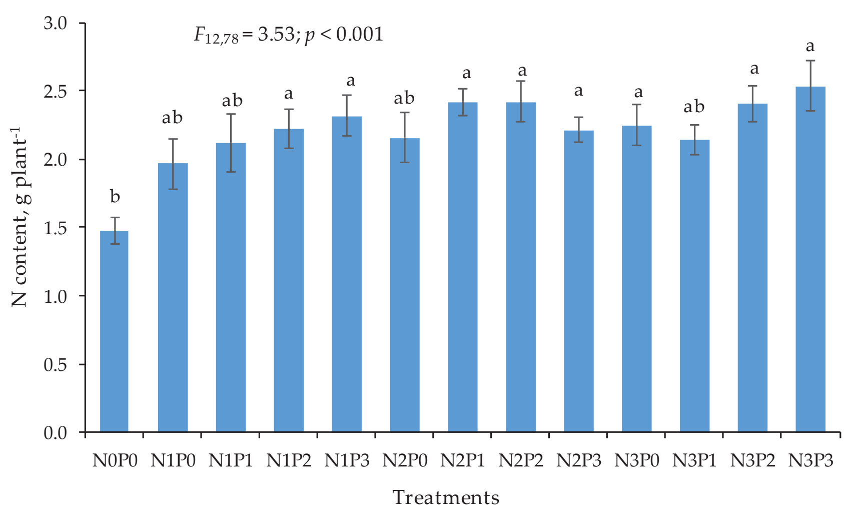

Figure 3. Content of nitrogen (N) in maize at the beginning of flowering depending on fertilization treatments. Two-year average values. Means within a column followed by the same letter indicate a lack of significant difference between the fertilized treatments (HSD test). Hatched bars represent 2 × SEM ranges.

2.5. Dry Matter and N Remobilization Indices

The effect of fertilization on the average values of indices describing the management of dry matter and N after flowering is shown in Table 4. The analysis of the growing season shows that the DMI and NI indices were higher in 2019 than in 2020. At the same time, a reverse interdependence was confirmed in relation to other indices. The values of DMR, DMRE and CDMR indices were negative in the first year of the study. In 2020, the values of the indices were positive. Such correlation resulted from a high growth of vegetative maize parts after flowering. In contrast to DM, the NI and NR indices were positive regardless of the year. NRE and CNR index values also confirm that nitrogen accumulated more in the green plant parts than in grain. The fertilization factor, however, had no significant impact on their values, nor did it exert any real influence in either year of the study. Nevertheless, some real trends emerged worth describing in terms of plants' reaction to the growing season and the NP(S) fertilization (Table S5). In this reference, as a result of the NP(S) application, the value index of NRE increased for N1 but only continued to grow up to the rate of P2. At the higher levels of nitrogen fertilization, the highest values were obtained in the treatments with a lower rate of P (N2P1) or without band application (N3P0), whereas the values of the CNR index were the highest for N1P0, N2P1, N3P0. The lowest CNR values were obtained in the N1P3, N2P3 and N3P1 treatments but without significant differences among treatments.

Table 4. Effect of the growing season and fertilization treatments on maize dry matter (DM) and nitrogen (N) management indices.

Year Treatment	Dry Matter Management Indices				Nitrogen Management Indices			
	DMA	DMR	DMRE	CDMR	NA	NR	NRE	CNR
	Effect of the year (Y)							
2019	165.6 ± 7.4 ^{a}	−50.4 ± 5.0 ^{b}	−61.5 ± 7.0 ^{b}	−40.6 ± 4.3 ^{b}	1.35 ± 0.12 ^{a}	0.70 ± 0.08 ^{b}	26.9 ± 3.0 ^{b}	39.7 ± 5.6 ^{b}
2020	93.8 ± 3.9 ^{b}	14.1 ± 2.9 ^{a}	11.3 ± 2.4 ^{a}	14.1 ± 2.9 ^{a}	0.82 ± 0.06 ^{b}	0.98 ± 0.05 ^{a}	44.7 ± 1.7 ^{a}	55.3 ± 3.0 ^{a}
	ANOVA results (significance level)							
Year	***	***	***	***	***	*	***	*
Treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Y × T	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

***, * significant at $p < 0.001$, $p < 0.05$, respectively; n.s.—non-significant; means within a column followed by the same letter indicate a lack of significant difference between the fertilized treatments (HSD test). DMI—dry matter increase; DMR—dry matter remobilization; NI—nitrogen increase; NR—nitrogen remobilization; DMRE—dry matter remobilization efficiency; CDMR—contribution of DMR assimilates to grain; NRE—N remobilization efficiency; CNR—contribution of remobilized N to grain N content.

In order to determine the interdependencies of the studied factors with the grain yield or the dry matter of the vegetative parts, a PCA analysis was carried out. Consequently, three factors were obtained, which, in combination, explained 94.6% of the total variance (Table S6). However, the first two, PC1 and PC2, explained 62.3% and 20.6% of the variance, which is equal to 82.9% of the total. Therefore, PC1 and PC2 were used to explain the interrelation between the analyzed parameters and the impact of the individual fertilization treatments on the axes representing particular variances (Figure 4). The indices of N nutrient management (CNR, NRE) were significantly, in a positive way, correlated with the DMRE and CDMR indices. The indices correlated more with GY than with DMRE and CDMR. The last two indices, however, negatively correlated with the content of N in plants at the flowering growth stage. That particular parameter, in turn, positively correlated with the dry matter of post-harvest residue and, interestingly, with the plants' dry matter at the seventh leaf stage. The values of correlation coefficients between the various features are included in the materials (Table S7).

The PCA biplot indicates the dominant impact of the seasonal factor on the axes arrangement of the interrelations mentioned above (Figure 4). As the analysis shows, most treatments were grouped near the Tukey median. Along the GY axis, quite a distance from the median, the following treatments were placed: control (N0P0) in 2019 and 2020, and the N1P0 treatment in 2020. On the opposite side of the median, the points representing different treatments were more scattered on both sides of the GY axis and, at the same time, the treatments creating clusters were more determined by the growing season than the fertilizer rate. On the left-hand side of the GY axis, therefore, for the high GY values, the treatments representing high fertilizer rates in 2020 were present (N2P2, N3P2, N3P3). For the lower GY values, on the right-hand side of the axis, various treatments from 2019 appeared, such as N2P1, N2P2 and N3P3. This result emphasizes the role of the growing season in the modification of the interdependencies among the treatments.

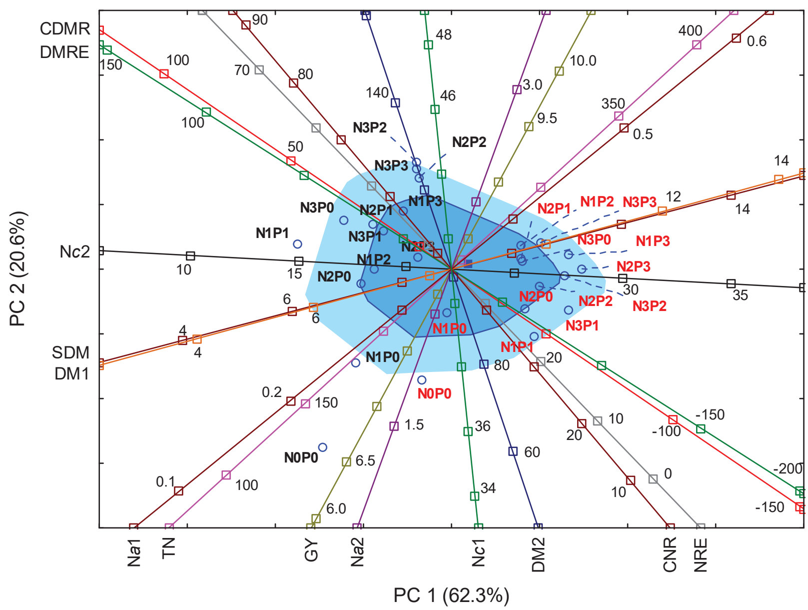

Figure 4. Principal component analysis (PCA) biplot of the maize yield, N content and accumulation, and N management indices. The dark blue square denotes the Tukey median, the blue square is the bagplot, the light blue square is the bagplot cover. Key: GY—grain yield; SDM—crop residues (straw) biomass; TN—total N accumulation; DM1—dry matter of plant at seventh leaf growth stage; N*c*1—N concentration at seventh leaf growth stage; N*a*1—N accumulation at seventh leaf growth stage; DM2—dry matter of plant at flowering; N*c*2—N concentration at flowering; N*a*1—N accumulation at flowering; DMRE—dry matter remobilization efficiency; CDMR—contribution of DMR assimilates to grain; NRE—N remobilization efficiency; CNR—contribution of remobilized N to grain N content. The treatments in 2019 are marked in red and the treatments in 2020 in black.

3. Discussion

The study reveals a lack of a significant impact of the growing season on grain yield. Maize requires between 500 to 800 mm of water depending on the environment [35]. In both growing seasons, the precipitation was approximately 250–300 mm. Taking into consideration the low water retention of the sandy soil and its water demand of 500 mm, it can be assumed that in the years of the study, water deficiency reached about 200–250 mm, and a potential grain yield loss could have reached 4–5 t ha^{-1} [36]. The stages of maize susceptible to water stress are the vegetative and reproductive stages, where yield loss may be as high as 18.6–26.2% and 41.6–46.6%, respectively [37]. The least favorable weather conditions during flowering were recorded in 2020. They were, however, compensated by heavy precipitation during the grain filling stage, whereas in 2019, the drought in June and early July occurred during the most intensive period for the plant. The negative effect of the drought on GY at that stage is manifested mainly in the reduction in plant height, leaf size and delay in leaf tip emergence [38]. Nevertheless, the drought may have also had a negative impact on the tasseling growth stage and successful pollination. As a result, GY was slightly higher in 2019 than in 2020.

A considerable difference was, however, recorded in the dry matter of post-harvest residues, as the vegetative growth was stimulated by heavy precipitation in 2019. Additionally, a high content of N_{min} in the soil in 2019 largely stimulated the development of vegetative biomass. Bearing in mind the variable weather and soil conditions, the influence of N and P(S) fertilization on the maize yield was quite similar over the years of the study; no statistically significant interaction was recorded. Nonetheless, at a higher level of nitrogen fertilization (N3 = 180 kg ha^{-1} of N), the degree of plant reaction to the band application of NPS depended on the year. In 2019, the yield-forming impact of the factor on GY and SDM was lower than in 2020, which resulted from a variable N_{min} content in the soil. The optimal N rate in maize cultivation depends on many factors. The most important ones include: soil type, the course of weather, water availability, N_{min} content in soil, as well as the time and method of nitrogen application [12,39,40]. According to the literature, N fertilization greatly increases GY, most often within the rate range of up to 120–220 kg ha^{-1} N [41–44]. Research carried out in northern China showed that the economically optimal N rate may be considerably higher and depend on the soil type [45]. According to the authors, it was 265 kg ha^{-1} of N in black soil, while in aeolian sandy soil, it was 186 kg N ha^{-1}. Ahmad et al. [46] point out that N applied to the soil in excessive amounts disturbs plant maturation and diminishes GY. In our own studies, the influence of the N rate on GY significantly depended on the ammonium phosphate fertilization level. On average, for the 2 years, the highest GY increase in comparison to the control was obtained for N2P3 (120 kg ha^{-1} of N) and N3P2 (180 kg ha^{-1} of N). It should, however, be emphasized that only slightly lower yield in comparison to the above-mentioned treatments was obtained for N1P3 (60 kg ha^{-1} of N). The difference was only 1.5–1.6%. Concurrently, the result indicates the dominant yield-forming impact of localized placement of NPS in comparison to the broadcast fertilization of N. In the present study, the optimal P rate depended on the level of N fertilization. For N1, the ideal rate was 26.2 kg ha^{-1} of P. For comparison purposes, during the broadcast application of P, the optimal rate in maize cultivation may reach even up to 100 kg ha^{-1} of P [47]. However, it should be stressed that the impact assessment of a single element P on maize in a ternary fertilizer mixture (N + P + S) is very difficult. Moreover, the fertilizer also included zinc (Zn). Both S and Zn have a positive effect on the uptake and metabolism of N in early growth, and consequently, on maize yielding [30,48]. The study hypothesis states, however, that the main components modifying the level of maize yielding and NUE indices are N and P. The hypothesis was based on the proven role of NH_4^+ and PO_4^{-3} ions in root formation, N uptake and, as a result, maize yield. The plants react much better to N in the form of di-ammonium phosphate (DAP) than to N with ammonium nitrate and urea applied, either before sowing, as fertilizer placement at sowing or at the fifth/sixth leaf growth stage [17,20,22]. Additionally, according to Weiß et al. [23], the starter fertilization DAP increases GY to a greater extent than a simultaneous application of triple superphosphate and ammonium nitrate. The result clearly indicates a synergistic effect of NH_4^+ and PO_4^{-3} ions. It is physically impossible to isolate the N or P effects in a binary fertilizer. The synergistic impact of N and P applied as DAP results from both specific transformations of NH_4^+ and PO_4^{-3} ions in soil, as well as their influence on root morphology [20]. Both ions feature low effective diffusion coefficients in soils [49]. The NH_4^+ ions readily bind to negative charges on the surface of clay minerals and become fixed [50], while PO_4^{-3} ions are readily fixed by adsorption to aluminum and other metal hydroxides or are precipitated depending on the pH as Fe-, Al- and Ca-phosphates [51]. According to Bordoli and Mallarino [27], P increased GY only in very low or low soil testing, and there was no response to P on any site. In our own study, despite high P concentration in the soil, P application may have had a positive impact on the maize yield, as the soil pH was acidic (5.2–5.3), and chemical sorption of P could have taken place [52]. Unlike broadcast fertilization, with or without soil incorporation, banding application reduces the surface area of contact with soil, thereby reducing PO_4^{-3} immobilization by fixation to various cations. Moreover, both ions stimulate the initiation and elongation of lateral roots on the part of the root system that is within or close to their respective nutrient depots, which is caused by the accumulation of the plant hormone auxin [53,54]. N and P also have a positive effect on root growth in soil zones distant from the nutrient patch [16]. In order to obtain a maximum maize yield, an early supply of adequate amounts of phosphorus to the plants is crucial [13]. Placing a NP mineral fertilizer near the maize seeds leads to a higher plant-available P concentration in the soil, greater uptake by plants and a higher unit production [15,22].

The content of nitrogen in the grain and in the post-harvest residue was higher in 2019 than in 2020. The result can be directly associated with the difference of N_{min} concentration in the soil. Fertilization treatments also exerted a great impact on the N content in grain. However, the experiment did not confirm any significant interrelation between the two factors. The average N content in grain was 17.9 and 16.3 g kg^{-1} of DM, depending on the year. According to Tenorio et al. [55], the grain of maize cultivated in the northcentral region of the USA ranged from 7.6 to 16.6 g kg^{-1}. Therefore, the N concentration obtained can be considered as being in the optimal range for maize grain. In addition, this level of Ng indicates a high efficiency of the soil (even on the control) as well as fertilization treatments. The high efficiency of N accumulation in grain was assured by high concentration of K and Mg in the soil [56]. Along with the N and P(S) rates, the N concentration increased, reaching the maximum value for the N3P3 treatment. The highest accumulation of TN (276.0 kg ha^{-1} of N) was also obtained for this treatment. It resulted from the stimulation of the highest NPS rates, N uptake and dry matter accumulation as a consequence of N absorption of solar radiation during plant maturation [57]. As Figure 3 shows, GY was positively correlated with TN. The regression equation for this correlation is as follows:

$$GY = 4.7591 + 0.0142 \times TN;\ R^2 = 0.72;\ p < 0.001;\ n = 26 \quad (1)$$

In order to assess the efficiency of nitrogen fertilization (NUE), several standard indices were used: partial factor productivity (PFP), agronomic efficiency (AE), partial N balance (PNB), apparent N recovery efficiency (RE), internal N utilization efficiency (IE) and physiological N efficiency (PE). Additionally, a relatively new index was chosen—nitrogen gap (N_{gap})—indicating potential yield losses as a result of imbalanced fertilization [32]. Among them, PFP, RE, IE and N_{gap} were considerably differentiated by fertilization factors. The values of the first three indices decreased as the N rate became lower. It is a general rule that the indices are higher with a low N rate [58]. PFP is a simple production efficiency expression, calculated in units of crop yield per unit of N applied. The advantage of PFP over others results from its sensitivity both to the course of weather and experimental factors. As the literature shows, the PFP values in maize cultivation for grain stay within the range of 6.1 and 114.9 kg kg^{-1} [14,44,59–61]. Thus, the PFP are within the above-mentioned range, yet they remain quite high. The result confirms the high productivity of the applied nitrogen. The experiment proved that PFP increased as the amount of NPS incorporated into the soil became higher and/or as the N share in the starter rate was greater. The differences were not significant but nevertheless clear for N1 and N2, while for N3, the differences were the smallest, clearly indicating the productivity decrease in high NPS rates when the maximum N rates were used. The other indices closely associated with the N rate—PNB and RE—point to the same trend. The expression of plant N content per unit of fertilizer N applied (PNB) indicates that, for N1, the maize effectively took advantage of the soil resources (the values were higher than 1). For N3, in turn, excessive N fertilization was recorded, expressed through values lower than 1 [62]. Regardless of the fertilization level, N exhibited a positive impact of NPS on the PNB values. With regard to RE, the index values for maize are, on average, 24.3–58.2% N [6,45,61]. Dhakal et al. [44] reported that the mean RE was 70% at the lowest N rate (60 kg ha^{-1} of N) compared to 50% at the highest N rate (240 kg ha^{-1} of N). The index is often used, as it measures the accumulation of the component in soil and/or the potential N losses to the environment [7]. In the present study, RE values were approximately 76.4% in 2019 and 60.8% in 2020. On average, for N1, N2 and N3, the index was 94.3, 63.7 and 47.7%. The use of NPS increased RE at every treatment level (and, at the same time, lowered potential losses of N to the environment), especially for the N1 treatment. For N1P3, the average value was >100%. The result can also be explained through the soil mining of N. A full confirmation of the hypothesis comes from the N_{gap} index. At the level of N1, negative values of N_{gap} were obtained and decreased along with the NPS rate. For N2 and N3, the positive values indicate the excess of N, which is the nitrogen not fully used by the plant. In the experiments, the AE index was also used to evaluate NUE. It reflects more closely the direct production impact of an applied fertilizer. Despite the fact that no statistically significant differences were obtained, a strong and clear trend of improving the index through the band application of NPS was noted. The trend was particularly transparent for N1 and then for N2. Typical AE levels of N for maize range from 1.9 to 29.0 kg grain kg^{-1} N [6,44,59,61,63]. Unlike this index, IE differentiated substantially between N and P(S) treatments. On average, over the two years of the study, lower values were recorded for N2P2 and N3P3 than for the control or for N1P0. The result indicates that 1 kg of uptaken nitrogen was more effective in terms of yield forming when NPS was being simultaneously applied. In summary, we can conclude that a shortage of P during the early growth stages results in a GY decrease, in turn negatively affecting NUE indices. The advanced procedure of NUE indices evaluation relies on the degree of their sensitivity to indicators of maize GY and N status at harvest. The PCA analysis revealed a number of interesting interrelations among the studied features. GY was positively correlated with TN, NHI and the NUE—IE index. The reliance of GY on IE and IE on NHI is described through the following equations:

$$GY = 12.004 - 0.1094 \times IE;\ R^2 = 0.42;\ p < 0.001;\ n = 26 \quad (2)$$

$$IE = -10.845 + 0.7819 \times NHI;\ R^2 = 0.55;\ p < 0.001;\ n = 26 \quad (3)$$

The increase in IE is associated with an increase in NHI, which in turn was associated with a higher N translocation efficiency in later stages of the grain filling period [31,60]. In our experiment, the NPS rate increase did not lead to the improvement of NHI. The plant response to this type of fertilization depended on the level of N fertilization.

The effect of applied fertilization treatments on NUE can be considered on many different levels. There are two stages that deserve special attention: (i) N uptake and accumulation in plants; (ii) remobilization of N from vegetative to generative parts. During the first stage, a phase of intense N accumulation in plants can be distinguished. It starts right after the sixth/seventh leaf growth stage and lasts until the beginning of maize flowering [64]. The optimal N content in maize at the sixth/seventh leaf growth stage ranges from 3.5 to 5.0 g kg^{-1} [65]. In our own study, the N content was therefore within the optimal range. Maize fertilized with NPS showed a higher concentration of that component than without NPS but only for the N1 and N2 treatments. The beneficial influence of NPS was particularly transparent for N1. As for N3, a positive impact of that factor was overshadowed by high rates of N. There are several possible explanations for the positive effect of banding NPS on N uptake and concentration. At the initial growth stage, this phenomenon can be explained by an increased N_{min} and P concentration in the soil solution near the roots [54]. At the same time, ammonium-induced acidification in the rhizosphere resulted in the increased solubility of sparingly soluble P compounds, such as apatite and struvite, resulting in higher P availability compared with the supply of NO_3-N [25,26]. Rhizosphere acidification induced by banding DAP application also increases the uptake of a greatly important element, namely Zn [17]. The authors also observed that there was a higher N uptake rate per unit of maize root biomass in response to band application of DAP compared with other treatments. The rise in the concentration of N in maize may also be explained through the modification of root architecture as a consequence of a high concentration of NH_4-N and P in the soil layer around the developing plants. Weligama et al. [21] report that band application increases total root length and root dry matter, while according to Ma et al. [17], it also positively conditions lateral root development. Ammonium only has a minor effect on root hair length or density, and an excess of phosphorus even reduces those features [66,67]. Earlier research additionally showed a beneficial effect of DAP application on maize N nutritional status at the sixth/seventh leaf stage, expressed by the plants' dry matter and SPAD index [14]. However, the influence depended on the depth at which the fertilizer granules were placed. In terms of N content, the optimum depth was 5–10 cm. At another critical growth stage, the beginning of flowering, localized NPS fertilization increased the total accumulation of N in plants. This was, among other factors, the consequence of better plant nutrition at an earlier stage. The amount of N at the flowering stage is particularly important, as after that stage, root activity and the ability to uptake N from the soil diminish [64]. According to Pampana et al. [34], before silking, maize uptakes approximately 64–70% of nitrogen from the soil. Most of the N in cereal kernels comes from the remobilization processes. The share of that form of N in kernels may constitute 50–90% of total nitrogen in cereal kernels [68,69]. As the main goal of maize cultivation was grain, therefore, the regression curves were determined to show the dependence of GY on N accumulation at the sixth/seventh leaf stage and flowering:

$$\text{6/7 leaf: GY} = 5.436 + 8.092 \times \text{Na1};\ R^2 = 56;\ p < 0.001;\ n = 26 \quad (4)$$

$$\text{Flowering: GY} = 5.14 + 1.401 \times \text{Na2};\ R^2 = 46;\ p < 0.001;\ n = 26 \quad (5)$$

In terms of plant nutrition diagnostics, the first interdependence is vital, because it is the last growth stage to change that in agricultural practice.

In order to determine the impact of N management on maize yielding, several indices were used. As far as the physiology of yielding is concerned, the following factors are considered largely relevant: dry matter remobilization efficiency (DMRE); contribution of DMR assimilates to grain (CDMR); nitrogen remobilization efficiency (NRE); contribution of remobilized N to grain (CNR). Earlier research confirmed that depending on the hybrid maturity class of maize, indices' values DMRE, CDMR, NRE and CNR can be 16.9–24.5%, 24.9–35.8%, 38.0–44.4% and 40.2–50.3%, respectively [34]. In comparison to these values, our own study obtained similar values for NRE and CNR. Nevertheless, DMRE and CDMR were negative. In 2019, the increase in the total maize biomass after anthesis (positive values of DMA) resulted mainly from the rise of the green parts of biomass. Thus, the DMR index was negative and, consequently, so were the DMRE and CDMR indices. This means that in 2019, the current photosynthesis was significantly responsible for the accumulation of DM in kernels. Such a reaction of plants is characteristic for the "stay green" types [5]. Ray et al. [33] also obtained negative results of DM remobilization indices as a consequence of large increases in maize biomass in the post-silking period. Unlike DM management indices, the nitrogen management indices (NRE and CNR) obtained in our studies were positive. These values confirm that N accumulation in kernels mainly relies on N remobilization. At the same time, in 2019, the remobilization index was considerably lower than in 2020, which should be associated with various concentration levels of N_{min} in the soil [5]. When maize N uptake is sustained throughout the grain filling period, less N is mobilized from vegetative organs, thereby increasing leaf area duration, delaying senescence and enhancing dry matter accumulation in grains [70]. In the conducted research, the fertilization factor changed the values of NRE and CNR indices in a particular way, usually making changes only up to a certain level. At the level of N1, the highest NRE value was obtained for the P2 rate, and at the level of N2, for the P1 rate, whereas for CNR, the optimum treatment was N2P1, followed by N1P0. The directions of the index value changes clearly indicate a competition between the main plant parts to obtain nitrogen and carbon, most probably determined by the plants' N nutritional status. This is confirmed by the lack of a positive correlation between the mentioned indices and the grain yield. As the earlier studies confirm, remobilization of N from vegetative plant parts was covered mainly by depletion of stem N at high N supply and by depletion of leaf N at low N supply [34,71]. If the main source of remobilized N was leaves, this brought about early leaf senescence and led to the decreasing accumulation of dry matter in grains from the current photosynthesis [72]. In the present studies, rising NPS rates boosted N accumulation in plants; therefore, the potential of utilizing N from leaves was high, but the real translocation of N to kernels was lower due to a higher metabolic activity of leaves well nourished in N and P(S). Our studies confirm earlier observations, where fertilization treatments without phosphorus (NK), indices of remobilization and contribution activities were higher than in treatments without nitrogen but with phosphorus (PK) [33]. At the same time, our studies indicate that N management indices do not have to have a linear correlation with NPS rates.

4. Materials and Methods

4.1. Experiment Location and Design

The experiment was carried out in 2019–2020 at Brody, Poznan University of Life Sciences Experimental Station, in Poland (52°26′18″ N 16°17′40″ E). The following factors were tested: band application of ternary fertilizer mixture (NPS) and different total N rates. Treatments of NP(S) fertilization were as follows: P_0—control; P_1—8.7; P_2—17.4; and P_3—26.2 kg ha^{-1} of P (17%, 33% and 67% of total P uptake for grain yield = 9.0 t ha^{-1}). The N rates were as follows: N_0—control; N_1—60; N_2—120; and N_3—180 kg ha^{-1} of N. A nitrogen rate of 180 kg ha^{-1} corresponded to 100% of maize requirement for this nutrient in mineral fertilizers. The effect of mineral fertilization with NP(S) was compared to the absolute control (AC), without fertilization with these components. NP(S) were applied to the soil using fertilizer NPS(+Zn) in the proportion 20-20-35+0.3 (calculated for N, P_2O_5, SO_3, Zn). The fertilizer is based on two main chemical compounds: di-ammonium phosphate, DAP [$(NH_4)_2HPO_4$] and ammonium sulfate [$(NH_4)_2SO_4$]. Therefore, on plots with localized fertilization P_1, P_2 and P_3, the doses of ammonium nitrogen (NH_4-N) were 20, 40 and 60 kg ha^{-1} of N. A detailed summary of the doses of N, P, S and Zn used in the experiment is given in Table 5. In order to simplify the notation of fertilization treatments, the NPSZn rates were recorded as P rates.

Table 5. Rates of nutrients (N, P, S and Zn) depending on the fertilization treatment.

Treatment	N (Band Appl. *) kg ha^{-1}	P kg ha^{-1}	S kg ha^{-1}	Zn g ha^{-1}
N0P0	0	0	0	0
N1P0	60 (0)	0	0	0
N1P1	60 (20)	8.7	14	300
N1P2	60 (40)	17.4	28	600
N1P3	60 (60)	26.2	42	900
N2P0	120 (0)	0	0	0
N2P1	120 (20)	8.7	14	300
N2P2	120 (40)	17.4	28	600
N2P3	120 (60)	26.2	42	900
N3P0	180 (0)	0	0	0
N3P1	180 (20)	8.7	14	300
N3P2	180 (40)	17.4	28	600
N3P3	180 (60)	26.2	42	900

* Nitrogen band application of di-ammonium phosphate (DAP) and ammonium sulfate.

The NP(S) fertilizer was applied while sowing the seeds. The fertilizer was banded to 5 cm below and away from the seeds. In treatments without NP(S) fertilization or with low total N rates, the maize requirement for N was supplemented with ammonium nitrate (34% N)—broadcast application immediately before sowing. Potassium fertilization was carried out in early spring in the form of potassium salt K(S, Mg) in proportion 41(15, 6.5) at the rate of 66.4 kg ha^{-1} of K, regardless of the NPS treatment.

A randomized complete block design (RCBD) with four replicates was applied in the experiment. The area of an individual plot was 24.0 m^2 (3 × 8 m). The variety *Zea mays* L. "ES Zizou" was used in the experiment (Euralis Semences, Lescar, France), which is a variety of FAO 220 earliness; type of seeds: flint-dent/flint; average early vigor and "stay green" type. Maize was also a forecrop. Sowing was performed on 20 April 2019 and 21 April 2020 with 95,000 seeds per hectare. The row spacing was 0.75 m, while the stem spacing was 0.14 m. Plant protection included the application of herbicide (mixture of terbuthylazine, S-metolachlor and mesotrione) before the emergence of the plants.

4.2. Soil and Meteorological Conditions

According to the World Reference Base for Soil Resources [73] classification system, the soil in the experiment was classified as Haplic Luvisols. The topsoil was characterized by sandy loam, and the subsoil was characterized by loam texture. The soil organic matter content in the topsoil was around 1.37%. To assess the pH and plant-available nutrient contents, soil samples were collected each year in early spring (March/April), before the application of mineral fertilizers. The soil pH at a depth of 0–0.3 m was acidic in each season. According to the Polish classification [74], the contents of plant-available P in the topsoil were high, irrespective of the year. The content of K in the first year was medium but in the second, very high. The content of Mg was very high; Ca—low; micronutrients—medium; and S—medium. The total content of N_{min} (sum of NH_4-N and NO_3-N in soil depth 0.0–0.9 m) ranged from 33.0 to 93.6 kg ha^{-1} of N (Table 6).

Table 6. Agrochemical properties of soil prior to experiment.

	Year/Soil Depth (m)					
	2019			**2020**		
	0.0–0.3	**0.3–0.6**	**0.6–0.9**	**0.0–0.3**	**0.3–0.6**	**0.6–0.9**
pH *	5.3	5.6	5.6	5.2	5.2	5.3
P, mg kg^{-1}	208.6	132.2	43.8	166.0	62.4	22.0
K, mg kg^{-1}	138.4	166.8	120.3	229.4	73.6	69.1
Mg, mg kg^{-1}	104.4	120.9	105.9	150.6	129.3	107.6
Ca, mg kg^{-1}	719.5	1523.3	1562.0	620.0	716.2	833.2
Cu, mg kg^{-1}	1.6	1.1	0.7	4.5	1.8	1.1
Fe, mg kg^{-1}	252.5	166.6	114.5	211.4	153.4	121.8
Mn, mg kg^{-1}	45.1	49.6	43.2	85.9	34.5	61.1
Zn, mg kg^{-1}	3.9	2.5	1.0	2.7	1.8	1.4
NH_4-N, kg ha^{-1}	10.9	4.1	9.0	7.2	0.6	0.9
NO_3-N, kg ha^{-1}	30.3	19.4	20.0	14.9	5.3	4.1
N_{min} sum, kg ha^{-1}	41.1	23.5	29.0	22.1	5.9	5.0

* soil pH was determined in suspension of 1 M KCl; plant-available nutrients using the Melich 3 method [75] and mineral nitrogen using 0.01 M $CaCl_2$ [76].

The characteristics of climatic conditions were based on data from the meteorological station belonging to the Poznan University of Life Sciences Experimental Station in Brody (west Poland). The long-term (1960–2018) average yearly precipitation and temperature in the study area are about 590 mm and 8.5 °C, respectively. In 2019 and 2020, the sum of precipitation was 403 and 496, and the average temperature 12.0 and 11.9 °C. In these years, the sum of precipitation during the growing season (April–September) was 253 and 313 mm, respectively. For comparison, the sum of precipitation for the long-term period was 356 mm. Both growing seasons also differed in their rainfall distribution. In the first year, May had better conditions for maize growth than in 2020. In turn, in the second year, the weather conditions were better in June and August. However, rainfall during flowering was greater in 2019 than in 2020 (Figure 5). Mean temperature during the maize growing season was 16.5 °C in 2019 and 15.7 °C in 2020.

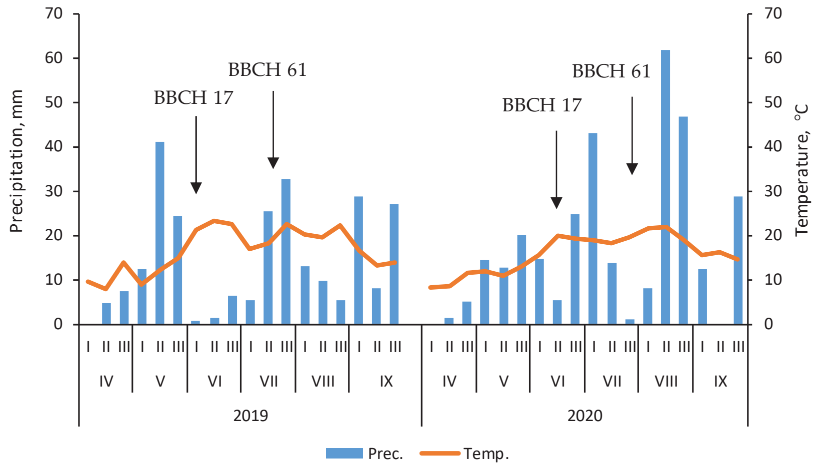

Figure 5. Air temperature and precipitation during 2019–2020 growing seasons of maize (in decades of the month). Key growth stages: BBCH 17—seventh leaf growth stage; BBCH 61—onset of flowering. Source: Poznan University of Life Sciences Experimental Station in Brody.

4.3. Plant Sampling and Analysis

Plant samples were collected at the following growth stages: seventh leaf (BBCH 17—coding system of growth stages, abbreviation (in German): Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie; [77]); beginning of flowering (BBCH 61/62) and technological maturity (BBCH 91/92). Depending on the year (2019 and 2020), the sampling dates were as follows: 8 and 16 June, 19 and 27 July, 27 September and 6 October. In order to obtain the grain yield, maize cobs were hand harvested from an area of 5.25 m^2 (2 rows × 3.5 m). Next, the cobs were counted and threshed using the laboratory threshing machine; the moisture of seeds was also determined. The biomass of plant vegetative parts was determined by randomly sampling 5 plants from each plot, regardless of the date of sample collection. The plant samples were dried at 60 °C and ground afterward. Nitrogen contents were determined with the Kjeldahl method using a Kjeltec Auto 1031 Analyzer (Foss Tecator AB, Hoganas, Sweden). Total nitrogen uptake (TN) was calculated by summing up the amount of nitrogen accumulated in grains (GN) and in the post-harvest residues (SN).

4.4. Nitrogen Use Efficiency Indices

In order to assess the effectiveness of nitrogen (N) fertilization, the following standard indices were used [62]:

$$\text{partial factor productivity of N (PFP)} = GY/N_f \ [\text{kg seeds kg}^{-1}\ N_f] \quad (6)$$

$$\text{agronomic efficiency of N (AE)} = (GY - GY_0)/N_f \ [\text{kg seeds kg}^{-1}\ N_f] \quad (7)$$

$$\text{partial N balance (PNB)} = TN/N_f \ [\text{kg N kg}^{-1}\ N_f] \quad (8)$$

$$\text{apparent N recovery efficiency (RE)} = (TN - TN_0)/N_f \ [\text{kg TN kg}^{-1}\ N_f] \quad (9)$$

$$\text{internal N utilization efficiency (IE)} = GY/TN \ [\text{kg seeds kg}^{-1}\ TN] \quad (10)$$

$$\text{physiological N efficiency (PE)} = (GY - GY_0)/(TN - TN_0) \ [\text{kg seeds kg}^{-1}\ TN] \quad (11)$$

where GY—grain yield (kg ha^{-1}); GY_0—grain yield in treatment without N application (kg ha^{-1}); N_f—input of N in mineral fertilizers (kg ha^{-1}); TN—total N accumulation in above-ground biomass of maize (kg ha^{-1}); TN_0—total N accumulation on control (kg ha^{-1}).

Additionally, the efficiency of N can be determined using the nitrogen gap (N_{gap}) index based on the concept proposed by Grzebisz and Łukowiak [32]. The following set of equations can be used to calculate N_{gap}:

$$\text{maximum attainable yield } (GY_{max}) = cPFP \cdot N_f \ [\text{kg ha}^{-1}] \tag{12}$$

$$\text{grain yield gap } (GY_{gap}) = GY_{max} - GY_a \ [\text{kg ha}^{-1}] \tag{13}$$

$$\text{nitrogen gap } (N_{gap}) = GY_{gap}/cPFP \ [\text{kg ha}^{-1}] \tag{14}$$

where cPFP is the third quartile (Q3) of the partial factor productivity (PFP) values measured for each plot with N fertilization (kg seeds kg^{-1} N_f); N_f—input of N in mineral fertilizers (kg ha^{-1}); GY_a—grain yield on each plot in a particular growing season (kg ha^{-1}).

4.5. Dry Matter and Nitrogen Management Indices

The assessment of the impact of fertilization on the method of dry matter (DM) and N management by maize was based on the following indices [68]:

$$\text{dry matter increase (DMI)} = DM_H - DM_A \ [\text{g plant}^{-1}] \tag{15}$$

$$\text{dry matter remobilization (DMR)} = DM_A - DM_S \ [\text{g plant}^{-1}] \tag{16}$$

$$\text{dry matter remobilization efficiency (DMRE)} = (DMR/DM_A) \times 100 \ [\%] \tag{17}$$

$$\text{contribution of DMR assimilates to grain (CDMR)} = (DMR/DM_G) \times 100 \ [\%] \tag{18}$$

$$\text{nitrogen increase (NI)} = TN_H - TN_A \ [\text{g plant}^{-1}] \tag{19}$$

$$\text{nitrogen remobilization (NR)} = TN_A - N_S \ [\text{g plant}^{-1}] \tag{20}$$

$$\text{nitrogen remobilization efficiency (NRE)} = (NR/TN_A) \times 100 \ [\%] \tag{21}$$

$$\text{contribution of remobilized N to grain (CNR)} = (NR/N_G) \times 100 \ [\%] \tag{22}$$

where DM_H—total above-ground biomass (dry matter) at maturity (g $plant^{-1}$); DM_A—total above-ground biomass at anthesis (g $plant^{-1}$); DM_S—dry matter of crop residues (stems, leaves, husk, corncob) at maturity (g $plant^{-1}$); DM_G—dry matter of grains at maturity (g $plant^{-1}$); TN_H—total N content of above-ground biomass at maturity (g $plant^{-1}$); TN_A—total N content of above-ground biomass at anthesis (g $plant^{-1}$); N_S—N content of crop residues (stems, leaves, husk, corncob) at maturity (g $plant^{-1}$); N_G—N content of grains at maturity (g $plant^{-1}$).

4.6. Statistical Analysis

The effects of individual research factors (year, NP treatments) and their interactions were assessed by means of the two-way ANOVA. If the F-ratio was larger than the critical value ($p < 0.05$), the differences between the treatments were evaluated by using the HSD (Tukey's) test (for $\alpha = 0.05$). The distribution of the data was checked using the Shapiro–Wilk test and the homogeneity of variance with the Bartlett test. Standard error of the mean (SEM) was used to indicate statistical error. Principal component analysis (PCA) was applied for evaluation of the relationships between variables. The Tukey median is surrounded by a bag containing 50% of the data points. The bagplot visualizes the location, spread, correlation, skewness and tails of data. The bagplot cover contains the inliers and outside of the "fence" are outliers [78]. In addition, the relationships between traits were analyzed using Pearson's correlation and linear regression. Statistica 13 software (TIBCO Software Inc., USA) was used for all statistical analyses [79].

5. Conclusions

The effect of nitrogen application on the maize yield and nitrogen management indices depended on the amount of mineral nitrogen in the soil, nitrogen doses and the method of its application. The most effective use of nitrogen by corn was ensured by the use of ammonium phosphate and ammonium sulphate during the sowing of corn seeds (band application). From the point of view of NUE indices, the optimal dose of N was 60 kg ha^{-1}. With broadcast fertilization and/or a further increase in the N dose, without the simultaneous use of P and S, the values of NUE indices deteriorated, especially in the year with the highest content of N_{min} in the soil. Thus, a positive effect of the interaction of N and P(S) was confirmed in the conditions of soil rich in plant-available phosphorus.

Band application had a particularly positive effect on total N accumulation, nitrogen harvest index and internal N utilization efficiency. These parameters were closely related to the grain yield. For diagnostic purposes, the accumulation of N in the above-ground part of maize at the sixth–seventh leaf stage was important.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants11131660/s1, Figure S1. Maize grain yield (GY), crop residues biomass (SDM) and total dry matter (TDM) depending on the year and fertilization treatments; Table S1. Maize yield components depending on the year and fertilization treatments; Table S2. Nitrogen concentration in dry matter (DM); accumulation of N in particular plant organs and total N accumulation by maize depending on the year and fertilization treatment; Table S3. Correlation matrix loadings and variance of the significant principal components (PCs) for grain yield of maize and nitrogen use efficiency (NUE) indices (n = 26); Table S4. Pearson correlation matrix between maize grain yield (GY), crop residues dry matter (SDM), total nitrogen accumulation (TN) and nitrogen use indices (n = 26); Table S5. Dry matter and nitrogen management indices depending on fertilization treatment; Table S6. Correlation matrix loadings and variance of the significant principal components (PCs) for grain yield of maize and dry matter and nitrogen management indices (n = 26); Table S7. Pearson correlation matrix between maize grain yield (GY), crop residues dry matter (SDM), total nitrogen accumulation (TN) and nitrogen content at early growth stages and N remobilization and contribution to grain (n = 26).

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Differentiation in Nitrogen Transformations and Crop Yield as Affected by Tillage Modes in a Fluvo-Aquic Soil

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Abstract: Nitrogen is a vital element for soil fertility and crop productivity. The transformation of nitrogen is directly affected by tillage practices for the disturbing soil. The characteristics of different nitrogen forms under different tillage modes are still unclear. A 3-year cycle tillage experiment was carried out to assess the combination of rotary tillage (RT), deep tillage (DT), and shallow rotary tillage (SRT) on nitrogen transformation and distribution, wheat yield and nitrogen balance in fluvo-aquic soil from Huang-Huai-Hai Plain in China. The results showed the rotation tillage cycle with deep tillage in the first year increased the total nitrogen (TN), and the main nitrogen form content in 0–30 cm compared with continued rotary tillage (RT-RT-RT). Moreover, the nitrate (NO_3^--N) and ammonium nitrogen (NH_4^+-N) content were improved in 20–40 cm by deep tillage practice with the highest value as 39.88 mg kg^{-1} under DT-SRT-RT. The time, tillage, and depth significantly affected the different nitrogen forms, but there was no effect on dissolved organic carbon (DON) and soil microbial biomass nitrogen (SMBN) by the interaction of time and tillage. Moreover, compared with RT-RT-RT, the rotation tillage promoted the spike number and kernels per spike of wheat, further increasing the wheat yield and nitrogen partial productivity, and with a better effect under DT-SRT-RT. The NO_3^--N and NH_4^+-N trended closer and positively correlated with wheat yield in 0–40 cm in 2019. The rotation tillage with deep tillage improved the different forms of nitrogen in 0–30 cm, wheat yield, and nitrogen partial productivity, and decreased the apparent nitrogen loss. It was suggested as the efficiency tillage practice to improve nitrogen use efficiency and crop yield in this area.

Keywords: rotation tillage; fluvo-aquic soil; wheat-maize system; nitrogen forms; nitrogen distribution

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1. Introduction

Nitrogen is the necessary element for the plant, which directly decides the crop yield [1]. The nitrogen transformation is affected by various factors, such as tillage, irrigation, fertilization, and so on. Tillage practice is a common agricultural practice to directly disturb and change the soil's physical properties, further affecting soil nutrient conversion and crop productivity [2]. The disturbance degree on soil varies from different tillage methods. Therefore, the effect of different tillage practices on soil physicochemical properties from different depths is different. Moreover, the tillage not only affects the change in soil nitrogen content, but also affects the profile distribution of soil nitrogen due to the downward shift of nitrogen and the effect of crop roots and may therefore influence crop yield and quality [3,4].

Soil nitrogen exists as organic components, its transformation in the soil is essentially associated with the interconversion of inorganic forms such as ammonium (NH_4^+-N), nitrate (NO_3^--N), and organic components [5], and is regulated by interactive processes of production and consumption [6]. The transformation process is driven by soil microorganisms. Soil microbial biomass nitrogen (SMBN) and dissolved organic nitrogen (DON) are important labile soil organic nitrogen (SON) fractions, which are considered actively involved in N mineralization and are potentially more sensitive indicators for agricultural management [7,8]. Although DON only accounts for 0.15–0.19% of soil TN, it is one of the relatively active components of the soil organic nitrogen pool and has important effects on nitrogen transformation and the environment. DON also represents a source of energy and nutrients for microbial growth and activity [9]. Tillage practice directly affects this process by changing the soil microenvironment, such as soil structure, and regulating the soil temperature and moisture, further mediating soil microorganism community and structure, and finally determining the nitrogen transformation. Studies have shown that conservation tillage usually increased soil N content compared to conventional tillage [2,3,10–12], but long-term no-till and reduced tillage produce nutrient accumulation and N mineralization in the soil surface [13], and may also cause topsoil compaction [14], leading to a reduction in the air-filled pore space [15,16], which may decrease the root absorption of soil nutrients and water [17]. Minimum tillage, often in combination with other practices, has been promoted to improve soil health through enhanced microbial activity and increased soil organic matter (SOM) in the surface layer [18–20]. long-term no-till management is a benefit for soil N stocks, N mineralization, and efficient fertilizer N use in corn-based cropping systems on well-drained soils. A meta-analysis by Mahal et al. [21] found that no-till increased potentially mineralizable N relative to moldboard plowing. Tillage can increase rates of soil C and N mineralization by disrupting aggregates and incorporating residue. Yuan et al. [6] found that no-tillage combined with maize straw mulching could simultaneously maintain the retention and availability of soil N to achieve effective N cycling in agroecosystems. Meanwhile, nutrients concentrated near the surface of no-till soils may increase the possibility of loss via erosion, runoff, and volatilization. Nutrient stratification may also reduce the availability and uptake of nutrients by crops.

In contrast, deep tillage can loosen the soil and promote crop roots grow and absorb more soil nutrients, but it may increase the loss of soil nitrogen and other nutrients, and accelerate soil erosion, cause serious environmental pollution and soil degradation thereby affecting ecosystem functions in the field [22,23]. Therefore, rotational tillage with the combination of different tillage practices was suggested to dismiss the disadvantage of long-term mono-conventional tillage [24]. Rotation tillage techniques that reasonably combined different tillage measures can effectively counteract some defects caused by mono-tillage practices [25–27]. In recent years, periodic disturbance of continuous NT systems in the form of occasional tillage, one-time tillage, strategic tillage, targeted tillage, single inversion tillage, one cycle of tillage, etc., has been promoted as a potential strategy to address the challenges of long-term NT management [28–31].

Huang-Huai-Hai Plain is the main agricultural region in China, with mainly wheat and maize double cropping systems. The long-term mono-rotary tillage with soil disturbance depth of around 15 cm in this area leads to a shallow plow layer and thick plow bottom, leading to the uncoordinated supply of soil water, fertilizer, gas, and heat, restricts the extension of crop roots, and impedes the increase in crop yields. Thus, optimum tillage practices are essential for crop production in this area. Therefore, the objectives of this study are (i) to clarify the differentiation in nitrogen transformation and distribution by the different tillage modes (ii) to assess the effect of different tillage modes on crop yield, nitrogen use efficiency, and nitrogen balance (iii) to select the optimum tillage mode according to the above results.

2. Results

2.1. Distribution of Soil Total Nitrogen under Different Tillage Modes

The total nitrogen content (TN) decreased with soil depth under all tillage treatments during 2017–2019 (Figure 1). The tillage affects the TN in the 0–40 cm soil layer in 2017 and the effect decreased within 0–30 cm in 2018 and 2019. TN content was no different in 0–10 cm and 40–50 cm in 2017 among the treatments, while it was significantly higher under the treatments with deep tillage combination in 20–40 cm than that under RT-RT-RT.

During 2018 and 2019, the deep tillage increased TN content in 0–30 cm soil layer compared with RT-RT-RT, with the higher one under DT-SRT-SRT and DT-SRT-RT as 1.14 g kg^{-1} and 1.13 g kg^{-1}, respectively, in 2018.

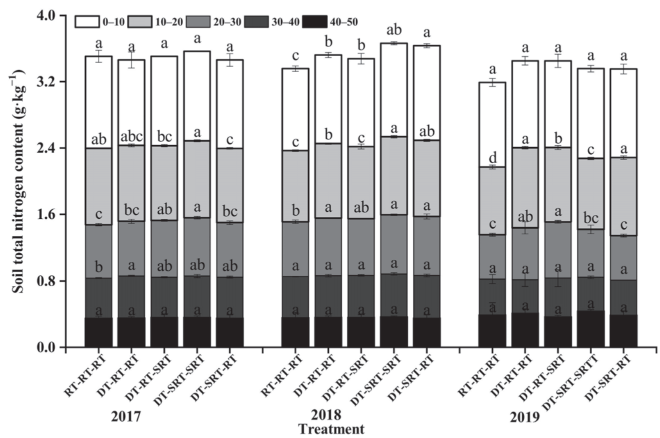

Figure 1. Soil total nitrogen content in different soil layers under different treatments. Note: Different lowercase letters indicate significant differences between different treatments at the same soil layer ($p \leq 0.05$).

2.2. Distribution of Soil Alkaline Nitrogen under Different Tillage Modes

Similar to TN, the alkaline nitrogen content (AN) under all treatments was decreased with the increase in soil depth during 2017–2019 (Figure 2). Compared with RT-RT-RT, the AN was affected by treatments with deep tillage in 0–40 cm in the first year (2017), and the effect decreased within 0–30 cm during the following two years. During the three-year experiment, the AN content was increased under the treatments with deep tillage compared with that under RT-RT-RT. While among the treatments with deep tillage, AN content did not demonstrate a significantly different or clear trend. It indicated that the effect of deep tillage was bigger than the combined other two tillage modes.

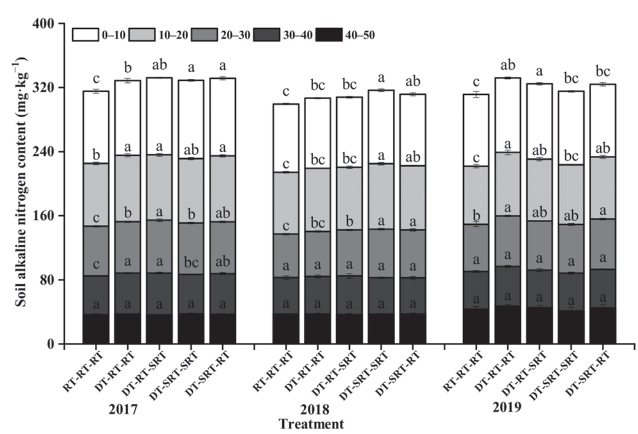

Figure 2. Soil alkaline nitrogen content in different soil layers under different treatments. Note: Different lowercase letters indicate significant differences between different treatments at the same soil layer ($p \leq 0.05$).

2.3. Distribution of Soil Nitrate Nitrogen under Different Tillage Modes

The nitrate nitrogen (NO_3^--N) content under all treatments was decreased with the increase in the soil depths, and the effect of tillage on NO_3^--N in 0–40 cm during 2017–2019 (Figure 3). In the first year, the NO_3^--N content in 0–20 cm soil layer under RT-RT-RT did not differ from that under the treatments with deep tillage, while, which was lower under RT-RT-RT in 0–40 cm during the following two years. Meanwhile, the NO_3^--N content in 30–40 cm soil layer under treatment with deep tillage increased with time, the NO_3^--N content under DT-SRT-SRT treatments was significantly higher than that under RT-RT-RT treatments, the highest increase was 35.12% in 2018. In 2019, the NO_3^--N content in the 0–40 cm soil layer was significantly increased under DT-SRT-RT with the highest value of 39.88 mg kg^{-1}. This indicated that the deep tillage accelerated the NO_3^--N leaching into the deeper soil layer.

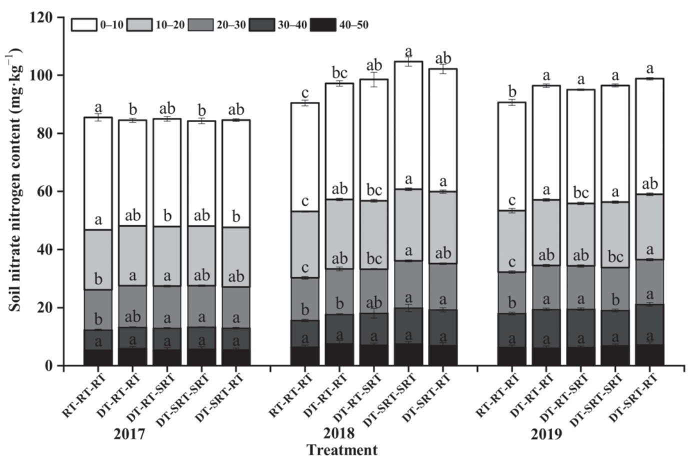

Figure 3. Soil nitrate nitrogen content in different soil layers under different treatments. Note: Different lowercase letters indicate significant differences between different treatments at the same soil layer ($p \leq 0.05$).

2.4. Distribution of Soil Ammonium Nitrogen under Different Tillage Modes

The ammonium nitrogen (NH_4^+-N) content under all treatments decreased with the increasing soil depths, and it slightly increased with time. The effect of tillage on NH_4^+-N in 0–40 cm during 2017–2019 (Figure 4). Similar to NO_3^--N, the NH_4^+-N content in 0–20 cm soil layer under RT-RT-RT did not differ from that under the treatments with deep tillage, while it was lower under RT-RT-RT in 0–40 cm during the following two years. Meanwhile, the NH_4^+-N content in 0–30 cm soil layer under treatment with deep tillage significantly increased with time, the NH_4^+-N content under DT-SRT-SRT treatments was significantly higher than that under RT-RT-RT treatments, the highest increase was 35.12% in 2018. In 2019, the NH_4^+-N content in the 0–40 cm soil layer was significantly higher under DT-SRT-SRT than that under RT-RT-RT with the highest value of 23.82 mg kg^{-1} in the 0–10 cm soil layer.

2.5. Distribution of Soil Dissolved Organic Nitrogen under Different Tillage Modes

The soil dissolved organic nitrogen content (DON) under all treatments decreased first and then slightly increased with time. The major change happened in the 10–30 cm soil layer (Figure 5). The DOC content did not differ from all treatments in 0–10 cm in 2017, but it decreased under RT-RT-RT in the following two years. The DOC was no different in the 40–50 cm soil layer in 2017 and 2018, while it was in the 30–50 cm soil layer in 2019. It indicated that the effect of deep tillage decreased in the third year. Although the

DON, generally, was no different among the treatments with deep tillage, the DON under DT-SRT-SRT and DT-SRT-RT was significantly higher than that under RT-RT-RT in 0–30 cm in 2018 and 2019, with the highest value of 23.37 and 25.49 mg kg^{-1} under DT-SRT-SRT in the 0–10 cm soil layer.

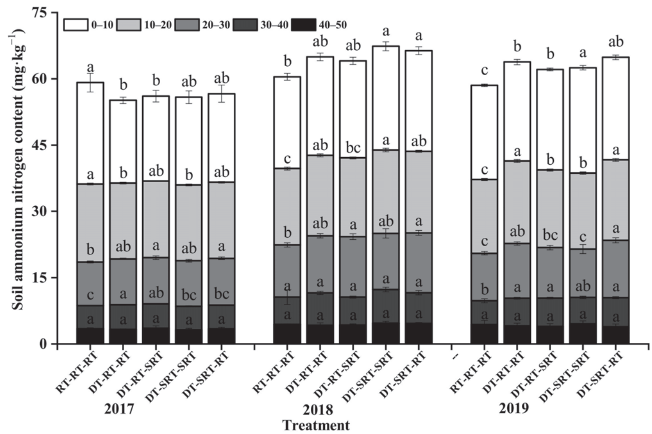

Figure 4. Soil ammonium nitrogen content in different soil layers under different treatments. Note: Different lowercase letters indicate significant differences between different treatments at the same soil layer ($p \leq 0.05$).

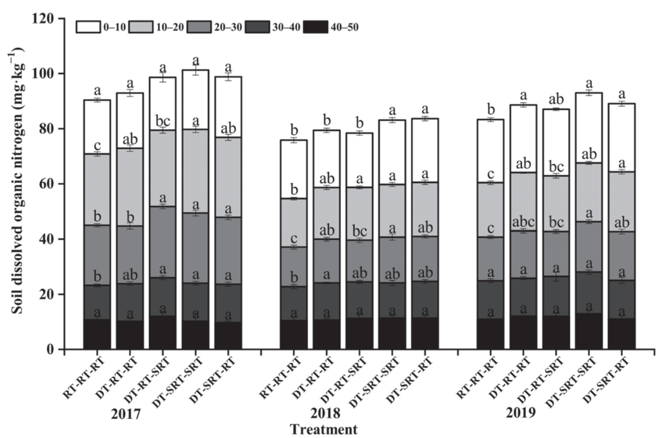

Figure 5. Soil dissolved organic nitrogen content in different soil layers under different treatments. Note: Different lowercase letters indicate significant differences between different treatments at the same soil layer ($p \leq 0.05$).

2.6. Distribution of Soil Microbial Biomass Nitrogen under Different Tillage Modes

The microbial biomass nitrogen content (SMBN) under all treatments decreased with soil depth increasing during 2017–2019 (Figure 6). Although the SMBN slightly fluctuated during the three years, it increased under the treatments with deep tillage compared with DT-SRT-RT in the 0–40 cm soil layer in 2017 and 2019, and in 0–30 cm in 2018. Generally, SMBN did not differ from the treatments with deep tillage in all the depths and years. The SMBN was significantly higher under DT-RT-SRT than under RT-RT-RT in the three-year experiment, with the highest values of 61.06, 63.03, and 63.26 mg kg^{-1} in 0–10 cm in the three years.

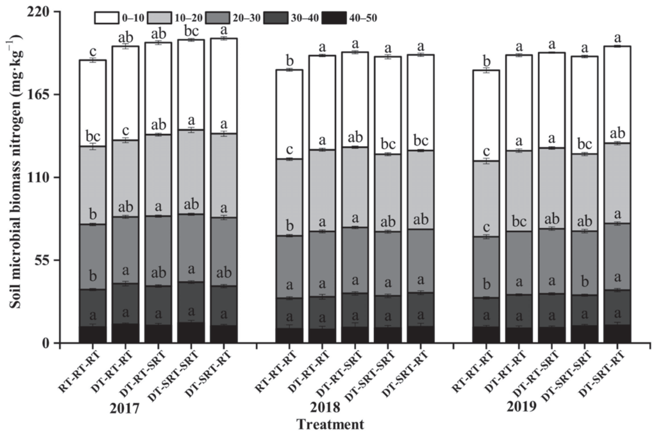

Figure 6. Soil microbial biomass nitrogen content in different soil layers under different treatments. Note: Different lowercase letters indicate significant differences between different treatments at the same soil layer ($p \leq 0.05$).

2.7. The Three-Factor Analysis with Time, Tillage, and Soil Depth on Nitrogen Forms

The multivariate analysis demonstrated that all nitrogen forms were affected by tillage time (year), tillage modes, and soil sample depth, respectively (Table 1). Therein, the effect of soil depth was the most important factor in the different nitrogen forms. All the nitrogen forms were affected by the interaction of tillage time and sample depth, tillage mode, and sample depth. However, there was no interaction effect on DOC and SMBN by tillage time and tillage mode.

Table 1. The three-factor analysis with time, tillage, and soil depth on different nitrogen forms.

Source of Variation	d.f.	TN	AN	DON	SMBN	NH_4^+-N	NO_3^--N
Time	2	15.2 **	89.73 **	237.79 **	34.77 **	127.87 **	290.33 **
Tillage	4	4.95 **	20.06 **	22.43 **	28.93 **	6.63 **	21.82 **
Depth	4	2340.33 **	6672.79 **	1218 **	11,731.24 **	6196.85 **	13,686.67 **
Time × Tillage	8	2.84 **	4.93 **	1.86 NS	1.08 NS	7.86 **	10.48 **
Time × Depth	8	8.7 **	37.96 **	152.92 **	18.12 **	10.06 **	35.38 **
Tillage × Depth	16	1.76 *	3.06 **	3.33 **	2.18 **	2.94 **	1.92 *
Time × Tillage × Depth	32	1.65 *	1.39 NS	1.85 **	2.15 **	3.01 **	3.01 **

The source of variation: "Time" means the tillage duration (year); "Tillage" means the five tillage modes; "Depth" means the five sample depths. * represented $p \leq 0.05$; ** represented $p \leq 0.01$; NS represented $p > 0.05$.

2.8. Wheat Yield, Yield Component, and Fertilizer Partial Productivity

The wheat yield, yield components, and nitrogen partial productivity changed over different years (Table 2). In the first year (2017), the spike number and thousand kernel weight did not differ from treatments, the difference in yield was driven by kernels per spike. The higher wheat yield was found under RT-RT-RT and DT-RT-RT with 6717 and 6383 kg ha^{-1}, respectively. In 2018 and 2019, generally, the wheat yield and yield component all showed higher under the treatments with deep tillage compared with RT-RT-RT. The highest wheat yield was under DT-SRT-RT in 2018 and 2019 with 6346 and 6557 kg ha^{-1}, respectively. The N partial productivity demonstrated a similar trend with wheat yield, with a higher value of 28.98 and 29.94 kg kg^{-1} in 2018 and 2019, respectively.

2.9. The Nitrogen Balance under Different Treatments

The nitrogen balance was calculated in 2019 (Table 3). Although the initial inorganic nitrogen under RT-RT-RT was the lowest one in all the treatments, the nitrogen absorbed by the crop was also the lowest one. The apparent nitrogen loss was the highest one with 46.11 kg ha^{-1}. This indicated that the rotation tillage modes helped to decrease the apparent nitrogen loss, and increase the nitrogen use efficiency.

Table 2. Wheat yield, yield components, and fertilizer partial productivity.

Year	Treatment	Spike Number ($\times 10^4$ ha^{-1})	Kernels Per Spike (No.)	Thousand Kernel Weight (g)	Yield (kg·ha^{-1})	N Partial Productivity
2017	RT-RT-RT	634.80 ± 29.55 a	29.53 ± 3.11 a	50.53 ± 2.41 a	6717 ± 103 a	30.67 ± 0.47 a
	DT-RT-RT	602.25 ± 26.25 a	28.83 ± 2.50 a	51.21 ± 1.20 a	6383 ± 208 ab	29.15 ± 0.95 ab
	DT-RT-SRT	586.35 ± 22.95 a	23.47 ± 3.06 b	49.68 ± 1.37 a	5967 ± 148 c	27.25 ± 0.68 c
	DT-SRT-SRT	600.75 ± 19.20 a	24.77 ± 2.67 ab	48.39 ± 0.94 a	6163 ± 191 bc	28.14 ± 0.87 bc
	DT-SRT-RT	598.50 ± 23.70 a	26.17 ± 2.04 ab	49.21 ± 1.60 a	6252 ± 277 bc	28.55 ± 1.27 bc
2018	RT-RT-RT	652.50 ± 31.50 c	25.33 ± 1.53 c	45.5 ± 0.68 a	6096 ± 148 c	27.84 ± 0.68 c
	DT-RT-RT	701.55 ± 20.55 ab	29.00 ± 1.73 ab	46.67 ± 2.04 a	6465 ± 102 ab	29.52 ± 0.47 ab
	DT-RT-SRT	721.35 ± 37.80 a	30.67 ± 2.08 a	45.97 ± 1.24 a	6507 ± 111 a	29.71 ± 0.51 a
	DT-SRT-SRT	677.55 ± 18.00 bc	26.00 ± 2.00 bc	47.72 ± 2.12 a	6259 ± 97 bc	28.58 ± 0.44 bc
	DT-SRT-RT	668.10 ± 16.35 bc	26.83 ± 1.04 bc	46.43 ± 1.50 a	6346 ± 79 ab	28.98 ± 0.36 ab
2019	RT-RT-RT	574.05 ± 12.75 d	28.43 ± 0.98 b	41.89 ± 1.028 c	5719 ± 153 d	26.12 ± 0.7 d
	DT-RT-RT	636.00 ± 8.85 b	28.93 ± 1.00 ab	43.36 ± 1.90 bc	6300 ± 53 b	28.77 ± 0.24 b
	DT-RT-SRT	604.35 ± 6.15 c	26.20 ± 1.80 c	48.57 ± 1.54 a	6003 ± 95 c	27.41 ± 0.44 c
	DT-SRT-SRT	648.30 ± 12.30 ab	30.80 ± 1.00 a	44.13 ± 1.20 bc	6477 ± 36 ab	29.58 ± 0.16 ab
	DT-SRT-RT	667.05 ± 8.10 a	31.00 ± 0.59 a	46.22 ± 0.95 ab	6557 ± 67 a	29.94 ± 0.31 a

Note: Different lowercase letters after the numbers indicate significant differences between different treatments ($p \leq 0.05$).

Table 3. The nitrogen balance under different treatments in 2019.

Treatment	Mineral Nitrogen (kg ha^{-1})	Initial Inorganic Nitrogen (kg ha^{-1})	Nitrogen Absorbed by the Crop (kg ha^{-1})	Residue Inorganic Nitrogen (kg ha^{-1})	Apparent Nitrogen Loss (kg ha^{-1})
RT-RT-RT	219	81.18 ± 0.79 c	173.39 ± 9.73 c	80.93 ± 1.40 c	46.11 ± 2.22 a
DT-RT-RT	219	83.23 ± 0.24 a	197.59 ± 4.40 ab	86.05 ± 0.89 a	18.62 ± 2.54 cd
DT-RT-SRT	219	82.83 ± 1.23 a	190.93 ± 10.26 b	85.55 ± 0.90 a	25.46 ± 2.95 b
DT-SRT-SRT	219	86.70 ± 0.80 bc	200.8 ± 5.02 ab	83.02 ± 0.87 bc	21.89 ± 1.97 bc
DT-SRT-RT	219	84.55 ± 0.73 ab	203.59 ± 3.22 a	84.03 ± 1.64 ab	15.93 ± 3.62 d

Note: Different lowercase letters after the numbers indicate significant differences between different treatments ($p \leq 0.05$).

2.10. The Correlation Analysis between Nitrogen Forms and Wheat Yield during 2017–2019

The correlation analysis found that the different nitrogen form in different soil layers was negative in the first year of the three-year rotation, while the correlation increased with time (Figure 7). In 2019, except TN and AN, the other nitrogen forms content was all significantly positively correlated with wheat yield in the 0–10 cm soil layer. Additionally, the NO_3^--N and NH_4^+-N were positively correlated with wheat yield in the 0–40 cm soil layer.

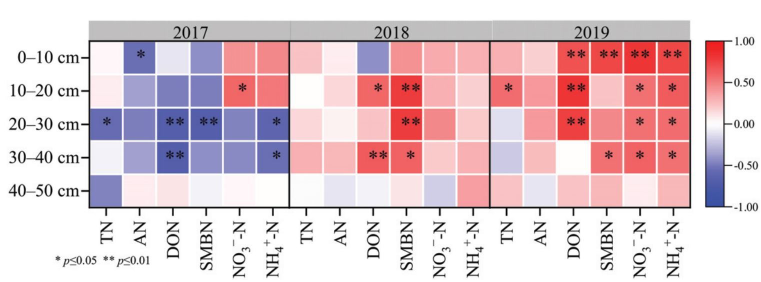

Figure 7. The correlation between nitrogen forms and wheat yield under different soil layers.

3. Discussion

3.1. The Effect of Tillage Practice on Total Nitrogen

Soil total nitrogen (TN) is the pool of nitrogen. It is one of the important indicators to assess soil fertility, but the major component of TN is organic nitrogen [32,33], which needs to transform into inorganic nitrogen such as nitrate nitrogen and ammonium nitrogen, to be absorbed by the crop. Soil tillage practices directly change the soil structure to improve the soil microenvironment [34], further mediating the nitrogen transformation process. Although the TN content is not sensitive to agricultural management, the different tillage practices change the TN vertical distribution by the different disturbance degrees of soil [35,36]. The TN content was changed in the 0–40 cm in the first year by different tillage practices, while the effect was decreased in the 0–30 cm during the following two years in this study. In addition, the effect of deep tillage mainly happened in 10–30 cm, and the combination tillage cycle with deep tillage increased TN content compared with RT-RT-RT. This might be because deep tillage helped to mix the surface soil and the deeper soil, the fertilizer, and the nutrient also mixed and provided the source of organic matter, which led to the TN content accumulation in the deeper soil layer [23,37]. The effect of deep tillage on soil nutrients and structure will decline with time [38]. Our results were in accordance with Han et al. [39] and Zhang et al. [23]. While Wang et al. [40] found that subsoiling—no tillage—subsoiling alternately could increase the TN content in the 0–20 cm soil layer, it did not affect the 20–40 cm soil layer. This might be because the soil disturbance by subsoiling was less than that by deep tillage, and the deep tillage takes more source of fertilizer or crop residue from the surface layer into the deeper soil layer [12,41].

3.2. The Effect of Tillage on Nitrogen Components

The nitrogen components were more sensitive than soil total nitrogen to tillage practices. Nitrate and ammonium nitrogen are the major inorganic nitrogen form in the soil, and they are also the main nitrogen form absorbed by the crop [42]. Their content is determined by the transformation between organic and inorganic nitrogen forms [5] and is regulated by interactive processes of production and consumption [43]. Soil microbial biomass nitrogen (SMBN) reflects the microorganism community, it is used to assess the nitrogen transformation process. Dissolved organic nitrogen (DON) is part of nitrogen that is relatively easy to transform. Tillage regimes impact the depth distribution of soil organic matter and affect the soil pore architecture which in turn influences soil aeration, and further regulates the nitrification, denitrification, and the relevant microorganic community and structure, finally affecting the NO_3^--N and NH_4^+-N, DON, and SMBN content [44,45]. Mondal et al. [46] reported that soil nitrogen status can be improved through no-tillage adoption particularly in the surface soil layer in a global meta-analysis. Minimum tillage, often in combination with other practices, has been promoted to improve soil health through enhanced microbial activity and increased soil organic matter (SOM) in the surface layer [18–20]. In contrast, deep tillage or deep subsoiling was conceived to break up the hard pan in farmland, eliminating soil compaction to boost plant root proliferation, penetration, nutrient uptake, and air permeability [38], improving biological health and physical properties of soil [47], facilitating rain infiltration and water retention [48], and hydraulic conductivity [49]. As a result, deep tillage accelerates the nitrogen transformation and distribution in different soil depths, meanwhile allowing the yield of crops to be continuously enhanced. Our study found that deep tillage promoted NO_3^--N and NH_4^+-N transportation into the deeper soil layer, especially for NO_3^--N. However, the effect of deep tillage on DON in deeper layers significantly declined with time. Although the deep tillage significantly increased the SMBN compared with RT-RT-RT, there was no significant change in the same soil layers with time. NO_3^--N cannot be fixed by soil colloid particles, and easily leach with soil water. Deep tillage promotes the soil pore and water storage capacity and helps the soil nitrification and NO_3^--N leaching [48,50]. For DON, although it is relatively easy to transform by the microorganism, there is still part of it belongs to organic form, this might the reason for the shorter affected by the deep tillage.

3.3. The Effect of Tillage on Wheat Yield and Nitrogen Balance

The tillage practices affect the soil structure and nutrient cycle, further regulating crop growth and yield [23]. Previous studies showed that although the no-till or minimal tillage profited to increase the soil nutrient in the surface soil layer [19,20,46], the effect on crop yield was different. Generally, no-till is considered shallow compaction or soil hardening by farm machinery traffic can lead to soil constraints to crop growth [51]. In contrast, most studies reported that deep tillage can increase crop yield by breaking up the hard pan in arable land and eliminating soil compaction to boost plant root proliferation, nutrient uptake, and air permeability [43]. As a result, deep tillage increases the plant availability of subsoil nutrients, which increases crop yield if nutrients are growth-limiting and allows the yield of crops to be continuously enhanced [43]. A similar result was found in this study, the wheat yield was increased under treatment with deep tillage compared with RT-RT-RT. Meanwhile, the nitrogen partial productivity demonstrated a similar trend with wheat yield. This indicated that deep tillage improved the nutrient absorbed by wheat and promoted the yield component and wheat yield. The correlation analysis also supported that it was a closer relationship between the wheat yield and nitrogen forms with time.

4. Materials and Methods

4.1. Site Description

The field experiment was carried out in 2016 at Yuanyang, Henan, China (35°19′ N, 113°50′ E). This area is a warm temperate continental monsoon climate. The mean annual air temperature is 14.5 °C, the mean annual precipitation is 615 mm, and the annual sunshine hours are 2324 h. The soil type is sandy fluvo-aquic soil developed from Yellow River alluviation, which is Calcaric Cambisol according to WBR [52]. The initial soil properties before the experiment in the 0–20 cm soil layer were: organic matter content 17.3 g kg^{-1}, total nitrogen 1.00 g kg^{-1}, alkaline nitrogen 71.33 mg kg^{-1}, available phosphorus 21.6 mg kg^{-1}, available potassium 108.0 mg kg^{-1}, pH 7.2. The field experiment was a winter wheat (*Triticum aestivum* L. Zhengmai 369)—summer maize (*Zea mays* L. Xundan 29) crop rotation.

4.2. Experimental Design

The randomized block design with three replicates was carried out. Five treatments with different combinations of tillage modes with three-year cycles were set as (1) continuous rotary tillage (RT-RT-RT); (2) deep tillage–rotary tillage–rotary tillage (DT-RT-RT); (3) deep tillage–rotary tillage–shallow rotary tillage (DT-RT-SRT); (4) deep tillage–shallow rotary tillage–shallow rotary tillage (DT-SRT-SRT); (5) deep tillage–shallow rotary tillage–rotary tillage (DT-SRT-RT). Each plot was 99.2 m^2. The information tillage practice before winter wheat seeding during 2016–2018 was demonstrated in Table 4.

Table 4. The soil tillage practice before winter wheat seeding during 2016–2018.

Treatment	10/2016	10/2017	10/2018
RT-RT-RT	rotary tillage	rotary tillage	rotary tillage
DR-RT-RT	deep tillage	rotary tillage	rotary tillage
DT-RT-SRT	deep tillage	rotary tillage	shallow rotary tillage
DT-SRT-SRT	deep tillage	shallow rotary tillage	shallow rotary tillage
DT-SRT-RT	deep tillage	shallow rotary tillage	rotary tillage

The tillage practice is detailed as follows. Summer maize straw was incorporated with all tillage practices. For rotary tillage, a rotary tiller was prepared twice with a depth of 13–15 cm. For deep tillage, first moldboard plows with 28–30 cm, then a rotary tiller was prepared twice with 15–18 cm. For shallow rotary tillage, a rotary tiller was prepared twice with 5–8 cm. The winter wheat was seeded by a seeder machine with a rate of 232.5 kg ha^{-1}. The basal fertilizer (N-P_2O_5-K_2O = 20-16-16) was applied 750 kg ha^{-1}, and then applied 69 kg N ha^{-1} at the regreening stage in the wheat season. The summer maize and fertilizer were seeded simultaneously with maize density as 67,500 plant ha^{-1} and 750 ha^{-1} component fertilizer (N-P_2O_5-K_2O = 28-10-12).

4.3. Soil Sample Collection and Measurement

The soil was sampled after the wheat harvest during 2017–2019. The 0–50 cm depth soil with 10 cm intervals was sampled by the mixture of 5–10 cores. The sample was divided into two parts, one part was stored at 4 °C in the refrigerator to determine soil nitrate nitrogen (NO_3^--N), ammonium nitrogen (NH_4^+-N), dissolved organic nitrogen (DON), and microbial biomass nitrogen (SMBN). The other part was air-dried and sieved through 0.85 mm and 0.25 mm to determine the soil alkaline nitrogen (AN) and total nitrogen (TN). The AN was measured by Conway method, and TN was determined by the micro-Kjeldahl method [53]. NO_3^--N and NH_4^+-N were extracted from 10 g of fresh soil in 50 mL of 2 mol KCl L^{-1} (1:10 soil: solution ratio) before filtering [54]. The NO_3^--N and NH_4^+-N concentrations in the extract were determined using an automated colorimeter (automatic chemical analyzer, Easychem Plus, Via Fratta Rotonda Vado Largo, Italy, Europe).

The dissolved organic nitrogen (DON) content was extracted using the method presented by Gigliotti et al. [55]. Briefly, 10 g of fresh soil with water at a soil-to-water ratio of 1:2 was shaken for 30 min h at 250 rev/min and 25 °C. Next, the supernatant was centrifuged for 10 min at 4000 rev/min before passing through a 0.45 μm membrane filter. The filtrate was measured using a TOC analyzer (Leeman, US17192017, Mason, OH, USA).

The soil microbial biomass nitrogen (SMBN) content was estimated using chloroform fumigation extraction according to the method presented by Vance et al. [56]. Briefly, 20 g of fresh soil was fumigated for 24 h at 25 °C with ethanol-free chloroform, the non-fumigated portion was completed simultaneously. Next, the soils were extracted using 60 mL of 0.5 mol K_2SO_4 L^{-1}, shaken at 200 rev/min for 30 min, and filtered using filter paper (12.5 cm diameter). The organic nitrogen contents in the extracts were determined using a TOC analyzer (Lehman US17192017). In addition, the SMBN content was calculated according to Jenkinson et al. [57]. as follows: microbial biomass nitrogen = E_N/k_{EN}, where E_N is the D-value between organic nitrogen extracted from fumigated soils and non-fumigated soils; k_{EN} = 0.45.

4.4. Grain Yield, Yield Components, Aboveground Biomass, and Nitrogen Accumulation

Three replicates of wheat samples (each 1 m^2) were randomly selected from each plot to measure yield components (spike number per hectare, grain number per spike, and 1000-grain weight) and nitrogen accumulation at the maturity stage. After threshing, drying, and weighing, wheat grain yield, and straw were calculated according to the national wheat grain and straw warehousing standard (at a moisture content of about 14%). The plant samples were oven dried (80 °C) over 48 h and weighed. The grain and straw were divided into two parts, and their nitrogen (N) content was analyzed using the micro-Kjeldahl method (Bao, 2000). Total aboveground nitrogen accumulation was calculated as the grain and straw N content, and the relevant biomass.

We used the certified standard reference materials (bush leaves, GBW07602 (GSV-1); soil, GBW07420), purchased from the National Center of Standard Material in China, to check the measurements.

4.5. Calculation

The nitrogen absorbed by aboveground biomass was calculated according to Lu et al. [58].

$$\mathrm{GNA} = \mathrm{GB} \times \mathrm{GNC} \tag{1}$$

$$\mathrm{SNA} = \mathrm{SB} \times \mathrm{SNC} \tag{2}$$

$$\mathrm{ANA} = \mathrm{GNA} + \mathrm{SNA} \tag{3}$$

where GNA was grain nitrogen accumulation (kg·ha^{-1}); GB was grain biomass (kg ha^{-1}); GNC was grain nitrogen content (kg kg^{-1}); SNA was straw nitrogen accumulation (kg ha^{-1});

SB was straw biomass (kg ha^{-1}); SNC was straw nitrogen content (kg kg^{-1}); ANA was aboveground nitrogen accumulation (kg ha^{-1}).

The apparent nitrogen loss (ANL) was calculated based on the ANA according to Xue et al. [59].

$$ANL = NI - NO \quad (4)$$

$$NI = IINS + NAR \quad (5)$$

$$NO = ANA + RINS \quad (6)$$

where ANL was apparent nitrogen loss (kg ha^{-1}); NI was nitrogen input (kg ha^{-1}), it was the sum of IINS and NAR; IINS was initial soil inorganic nitrogen storage (kg ha^{-1}) in the 0–20 cm soil layer; NAR was nitrogen application rate in wheat season (kg ha^{-1}); NO was nitrogen output (kg ha^{-1}), it was the sum of ANA and RINS (kg ha^{-1}); ANA was aboveground nitrogen accumulation (kg ha^{-1}); RINS was residue inorganic nitrogen storage (kg ha^{-1}) in the 0–20 cm soil layer after wheat harvest in 2019.

4.6. Statistical Analysis

Microsoft Excel 2020 (Microsoft Corp., Redmond, WA, USA) was used to input and organize the data, using SPSS Software (ver. 20.0; SPSS Inc., Chicago, IL, USA) for statistical analysis. The ANOVA analysis was used to compare the difference in different nitrogen forms, grain yield, yield components, nitrogen partial productivity, and nitrogen balance indexes between different tillage modes. The multiple comparisons by the least significant range method (LSD) were to analyze the effect of tillage mode, soil depth, and tillage time on the different nitrogen forms. Origin Pro (ver. 8.5; OriginLab Corporation, Northampton, MA, USA) was used to create the graph. All statistical analyses were performed at a significance level of $p \leq 0.05$.

5. Conclusions

The findings carried out from the 3-year cycle tillage experiment showed that the rotation tillage with deep tillage in the first year increased the total nitrogen and the major nitrogen forms content compared with RT-RT-RT. Especially they improved the NO_3^--N and NH_4^+-N content in 0–40 cm, with the highest value under DT-SRT-RT. The time, tillage, and depth significantly affected the different nitrogen forms, but there was no effect on DON and SMBN by the interaction of time and tillage. Meanwhile, the rotation tillage promoted the spike number and kernels per spike of wheat, further increasing the wheat yield and nitrogen partial productivity, and with a better effect under DT-SRT-RT. The available nitrogen forms such as NO_3^--N, and NH_4^+-N were closely positively correlated with wheat yield in 0–40 cm at with time.

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plants

Article

Optimizing Nitrogen and Seed Rate Combination for Improving Grain Yield and Nitrogen Uptake Efficiency in Winter Wheat

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Abstract: Nitrogen (N) supply and seed rate (SR) are two essential factors that affect the accumulation and partitioning of N and dry matter (DM) and, therefore, grain yield (GY) and N use efficiency (NUE). The objective of this experiment was to optimize N application and SR to regulate wheat growth and increase both GY and NU_E. The results revealed that net photosynthetic rate (Pn), stomatal conductance (Gs), chlorophyll content, and activities of metabolic enzymes (NR and GS) significantly increased with increasing of N levels while decreasing SR. Plant tillers, GY, DM before anthesis, and N translocation, N agronomic efficiency (NA_E), N recovery efficiency (NR_E), and N uptake efficiency (NUP_E) were highest in a combined treatment of N_{235} and SR_{180}. However, N levels beyond 235 kg ha^{-1} significantly decreased NA_E, NR_E, and NUP_E. By increasing SR from 135 to 180 kg ha^{-1} an increase of 12.9 % and 9.1% GY and NUPE, respectively, was observed. Based on this result, we estimate that 1 kg N ha^{-1} might be replaced by an increase of approximately 0.6 kg ha^{-1} SR. Our study suggested that using a combination of N and SR (N_{235} + SR_{180}) could attain maximum GY and improve NU_E parameters.

Keywords: high yield; nitrogen application; N use efficiency; seed rate; winter wheat

1. Introduction

Wheat is the main staple crop globally and plays a crucial role in challenging food security, with a total production of 736.1 million tons. Wheat grain yield is not only dependent on genetic potential (variety) and environmental constraints [1] but also depends on management practices [2,3]. Nitrogen is the essential nutrient for wheat growth and production [4,5] which is necessary for maintaining plant growth, biomass, and grain yield [6]. N deficiency in cereal crops reduces fertile tiller numbers [7–9], grain number, and kernel weight [10,11]. However, overuse of N results in environmental problems including N leaching, runoff, and volatilization [12], and reduces overall N use efficiency (NU_E) [13]. In China, a rapid increase in wheat yield was in parallel with the dramatic use of N fertilizer since the 1950s [14]. Importantly, in the last 20 years, N input beyond the threshold level only caused prolonged yield improvement while severe environmental pollution [12,15,16]. Thus, the China government proposed the Double Reduction Plan [15]. A low dose of N with high NU_E must be one of the main research goals in plant nutrition [16].

However, enhancing crop profitability and NU_E simultaneously is necessary for sustainable agriculture [17] and it is a key challenge to improve viable agriculture in the next decades [18]. N fertilizer is often widely overused to obtain ideal productivity while the adjustment of SR is neglected, which usually synchronously improves productivity and NU_E. When the N rate was decreased or cut, the output would be lost by reducing tillering and fertile tillers, grain number, and kernel weight [19]. The increase in SR could partially compensate for the decrease in fertile tillers and spike numbers and final productivity [20]. Increasing plant density from 135 to 405 plants m^{-2} [21] or 75 to 300 plants m^{-2} [11] significantly increased grain yield and other parameters. However, there must be an optimum SR to compensate for the negative effects of decreasing N for balanced high yields and improved NU_E in wheat. Therefore, it is necessary to investigate the compensatory effect of increasing SR on the decreasing N input on wheat productivity and N use efficiency. It is also necessary to clarify their combination effects on the physiological and agronomical performance of wheat to reveal the underlying rules for balanced high grain yield and improved NU_E in winter wheat.

A field experiment with different N and SR levels in two successive years was carried out to determine the optimum combination of N and the seed rate leading to improvement in grain yield and N use efficiency.

2. Results

2.1. Physiological Traits

2.1.1. Photosynthetic Capacity, Chlorophyll Content, and Leaf Area Index

Pn, Gs, SPAD, and LAI were highest at the anthesis stage, followed by the jointing stage, 10 days after anthesis (10 DAA) and 20 DAA, respectively. N application and SR have significant effects on various physiological traits, viz., leaf photosynthetic capacity, chlorophyll content (SPAD value), as well as leaf area index (LAI) at all growth stages (Figures 1–3). Photosynthetic capacity (Pn), stomatal conductance (Gs), and SPAD were significantly increased by increasing the N rate or decreasing SR. A significant increment was observed as the N application rate increased from 0 to 235 kg ha^{-1}. When N increased from 235 to 290 kg ha^{-1}, there was no significant increase for Pn, Gs, and SPAD in both growing seasons. The Pn, Gs, and SPAD values were decreased significantly when SR increased from 135 to 225 kg ha^{-1} in both growing seasons at all sampling stages. Moreover, LAI was significantly increased with an increase in both factors (N + SR). A significant effect was observed when N increased up to N_{235} treatment. There was no significant difference between the N_{235} and N_{290} treatments in LAI in both growing seasons (Figure 3). SR also significantly increased the LAI in both growing seasons from SR_{135} to SR_{180}, beyond this level there was no significant effect.

2.1.2. Enzymatic Activities of NR and GS

Nitrate reductase (NR) and glutamine synthesize (GS) play an essential role in N metabolism assimilation and regulation. NR and GS′s enzyme activities were significantly increased by increasing N and decreasing SR (Figure 4). However, the activities of NR and GS differed at the various levels of N application. Furthermore, there was a significant variation in NR and GS activities between N_0, N_{180}, and N_{235}, whereas there was no significant difference between the N_{235} and N_{290} treatments. The highest value of the NR and GS activities appeared as the combination of N_{290} and SR_{135} treatments (Figure 4).

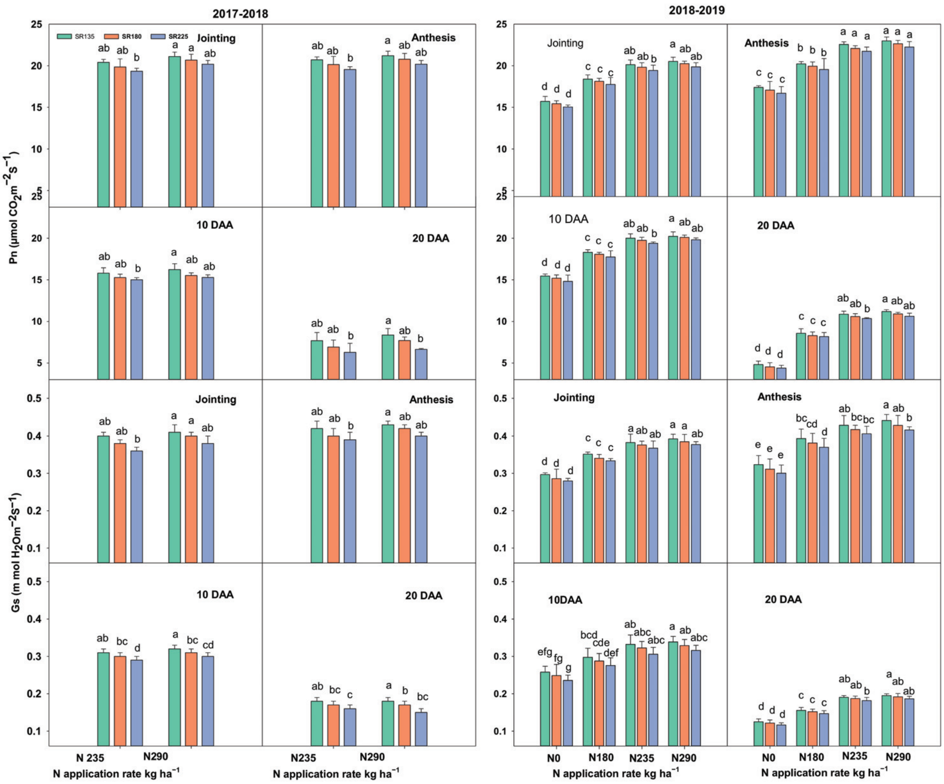

Figure 1. Effects of the combination of N and SR on photosynthesis (Pn) and stomatal conductance (Gs) during four differnet growing stages in 2017–2018 and 2018–2019. Different letters represent significant differences in mean values of three replicate plots at $p \leq 0.05$ levels according to Duncan's test.

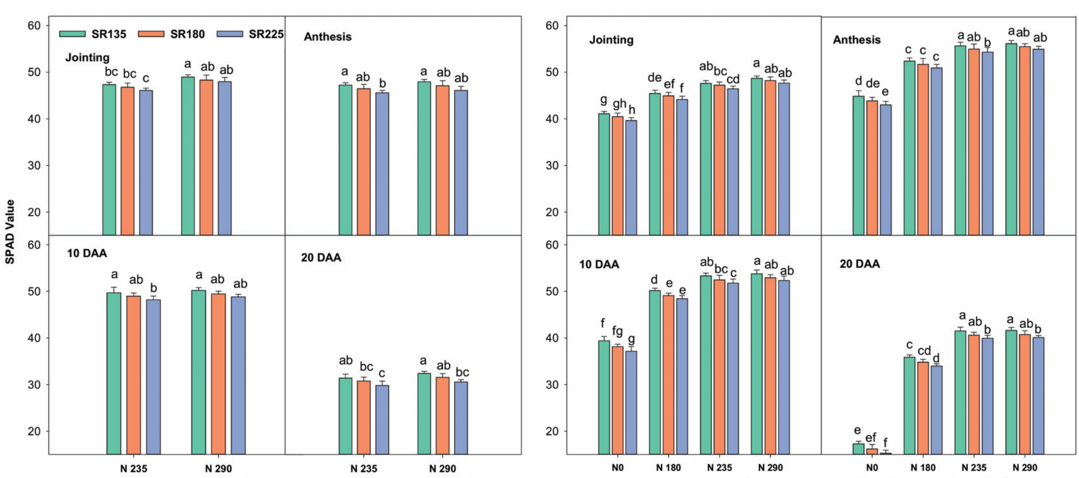

Figure 2. Effects of N and SR on chlorophyll content (SPAD) during four differnet growing stages in 2017–2018 and 2018–2019. Different letters represent significant differences in mean values of three replicate plots at $p \leq 0.05$ levels according to Duncan's test.

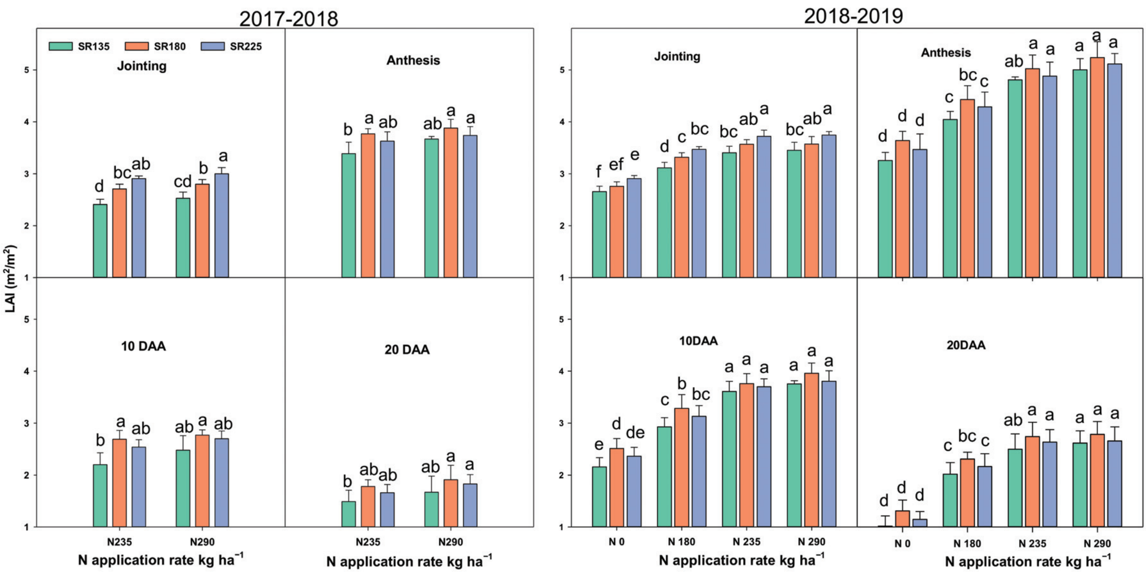

Figure 3. Effects of the combination of N and SR on the values of the leaf area index (LAI) during four differnet growing stages in 2017–2018 and 2018–2019. Different letters represent significant differences in mean values of three replicate plots at $p \leq 0.05$ levels according to Duncan's test.

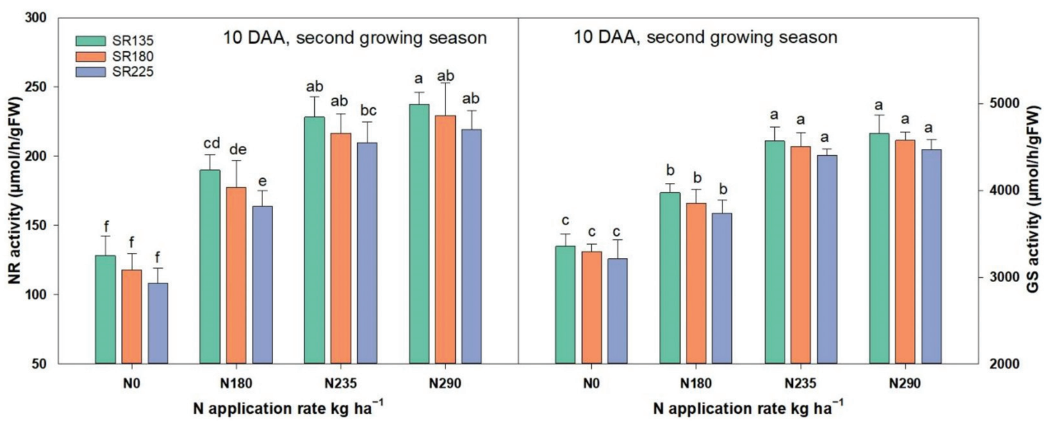

Figure 4. Combination effects of N and SR on nitrate reductase (NR) and glutamine synthesize (GS) activities during four differnet growing stages in 2018–2019. Different letters represent significant differences in mean values of three replicate plots at $p \leq 0.05$ levels according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

2.2. Grain Yield and Related Agronomic Characteristics

The grain yield (GY) was significantly influenced by N level and seed rate (N and SR), as well as by their interaction in both years (Table 1). With the increase in the N application rate the GY significantly increased. At SR of 180 kg ha^{-1}, GY increased from 6.7 to 8.3 t ha^{-1} when N application increased from N_{180} to N_{235} kg ha^{-1} in 2018–2019. There was no significant difference between N_{290} and N_{235} kg ha^{-1} in both growing seasons. SR significantly affected GY in both growing seasons. Under the same amount of N application (N_{235} kg ha^{-1}), GY increased from 5 to 5.7 t ha^{-1} in the first growing season and from 7.5 to 8.3 t ha^{-1} in the second growing season (Table 1). The highest GY 8.3 t ha^{-1} was obtained with the combination of N_{235} + SR_{180} kg ha^{-1} in 2018–2019. N and SR significantly affected the agronomic parameters viz., number of spikes (NS), 1000-grain weight (TGW), number of grains per spike, harvest index (HI), and plant height (PH). All agronomic parameters (NS, TGW, NGS, HI, and PH) were significantly increased with the increasing N application rate. Compared to the treatment with SR_{180} N_0, NS was significantly increased by 13.2%, 23.8%, and 22.4 % and NGS was increased by 21.8%, 36.8%, and 37.8% in treatments $SR_{180}N_{180}$, $SR_{180}N_{235}$, and $SR_{180}N_{290}$, respectively, in the second growing season. Except for the first growing season in which PH was scarcely increased, TGW and HI were decreased when N application rate increased from N_{235} to N_{290}. At the second growing season, none of the agronomic characteristics were significantly influenced beyond N_{235} kg ha^{-1}.

Table 1. Effects of N and SR on GY and agronomic characteristics in 2017–2018 and 2018–2019.

	N kg ha^{-1}	SR kg ha^{-1}	TY t ha^{-1}	NS × 10^4 ha^{-1}	TGW(g)	NGS	HI	PH (cm)
2017–2018	N_{235}	SR_{135}	5.05 ^{b}	357 ^{c}	39.7 ^{a}	35.63 ^{a}	0.41 ^{c}	73.4 ^{c}
		SR_{180}	5.78 ^{a}	414 ^{b}	39.5 ^{ab}	35.38 ^{a}	0.44 ^{ab}	74.33 ^{bc}
		SR_{225}	5.7 ^{a}	425 ^{ab}	39.2 ^{bc}	34.27 ^{b}	0.45 ^{a}	75.74 ^{ab}
	N_{290}	SR_{135}	5.03 ^{b}	361 ^{c}	39.1 ^{bc}	35.63 ^{a}	0.41 ^{c}	76.43 ^{ab}
		SR_{180}	5.77 ^{a}	420 ^{ab}	39 ^{bc}	35.3 ^{a}	0.43 ^{bc}	77.23 ^{a}
		SR_{225}	5.77 ^{a}	434 ^{a}	38.9 ^{c}	34.18 ^{b}	0.42 ^{bc}	77.57 ^{a}
	F-Value	N	0.113	2.475	13.65 **	0.098	7.478 *	19.88 **
		SR	107.1 **	105.9 **	3.38 *	21.65 **	11.46 **	2.999
		N*S	0.331	0.123	0.63	0.025	1.637	0.426
2018–2019	N_0	SR_{135}	4 ^{g}	391 ^{g}	35.7 ^{bc}	28.8 ^{d}	0.35 ^{e}	62.93 ^{d}
		SR_{180}	4.8 ^{f}	479 ^{ef}	35.6 ^{bc}	28.1 ^{d}	0.4 ^{cde}	63.62 ^{d}
		SR_{225}	4.8 ^{f}	503 ^{de}	35.5 ^{c}	27.2 ^{d}	0.4 ^{cd}	64.07 ^{d}
	N_{180}	SR_{135}	5.6 ^{e}	448 ^{f}	36.4 ^{ab}	34.5 ^{c}	0.38 ^{de}	72.87 ^{c}
		SR_{180}	6.7 ^{d}	543 ^{bc}	36.3 ^{abc}	34.1 ^{c}	0.41 ^{cd}	74.02 ^{bc}
		SR_{225}	6.6 ^{d}	555 ^{b}	36.3 ^{abc}	33.1 ^{c}	0.42 ^{bc}	74.47 ^{b}
	N_{235}	SR_{135}	7.5 ^{c}	518 ^{cd}	36.7 ^{a}	39.6 ^{ab}	0.45 ^{ab}	73.97 ^{bc}
		SR_{180}	8.3 ^{a}	593 ^{a}	36.7 ^{a}	38.3 ^{ab}	0.46 ^{a}	75.15 ^{ab}
		SR_{225}	8.2 ^{a}	597 ^{a}	36.5 ^{ab}	37.5 ^{b}	0.46 ^{a}	75.75 ^{a}
	N_{290}	SR_{135}	7.8 ^{bc}	529 ^{bc}	36.6 ^{a}	40.4 ^{a}	0.45 ^{ab}	73.95 ^{bc}
		SR_{180}	8.2 ^{a}	587 ^{a}	36.4 ^{ab}	38.6 ^{ab}	0.44 ^{ab}	75.22 ^{ab}
		SR_{225}	8.1 ^{ab}	591 ^{a}	36.4 ^{ab}	37.7 ^{ab}	0.45 ^{ab}	75.97 ^{a}
	F-Value	N	690 **	74.4 **	9.66 **	242.5 **	43.55 **	598.3 **
		SR	60.1 **	83.94 **	0.66	12.19 **	4.48 *	17.8 **
		N*SR	2.64 *	1.36	0.027	0.394	1.77	0.25

Note: N, SR, TY, NS, TGW, NGS, HI, and PH indicate nitrogen application, seed rate, theoretical yield number of spikes, thousand-grain weight, the number of grains per spike, harvest index, and plant height, respectively. Different letters in the same column represent significant differences in mean values of three replicate plots according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

SR significantly influenced all of the agronomic parameters. Increasing SR significantly increased NS, HI, and PH during both growing seasons and decreased NGS and TGW. As an example, compared with the $N_{235}SR_{135}$ treatment, NS was significantly increased by 10.8% and 11.6%, while NGS was decreased by 4.5% and 6.7% in treatments $N_{235}SR_{180}$ and $N_{235}SR_{225}$, respectively, in the second growing season (Table 1).

Increasing the application of N increased the fodder part and the yield of wheat. Increasing the seed rate can increase the spike number and compensate for reducing the N application rate. Linear regression was used to assess replacing N with SR for balancing GY and NU_E parameters. According to the equations obtained from linear regression, the increase in grain yield by 1 ton ha^{-1} needs to increase the seed rate by 7669 kg ha^{-1} or increase the fertilization with N by 12,807 kg ha^{-1}. This means that 7669 kg ha^{-1} seed rate is equivalent to 12,807 kg N; therefore, 0.598 kg ha^{-1} seed rate would approximately replace 1 kg of N ha^{-1} (Figure 5). Based on our result, increasing SR from 135 to 180 kg ha^{-1} was observed to increase by 12.9% and 9.1% for GY and NUPE, respectively.

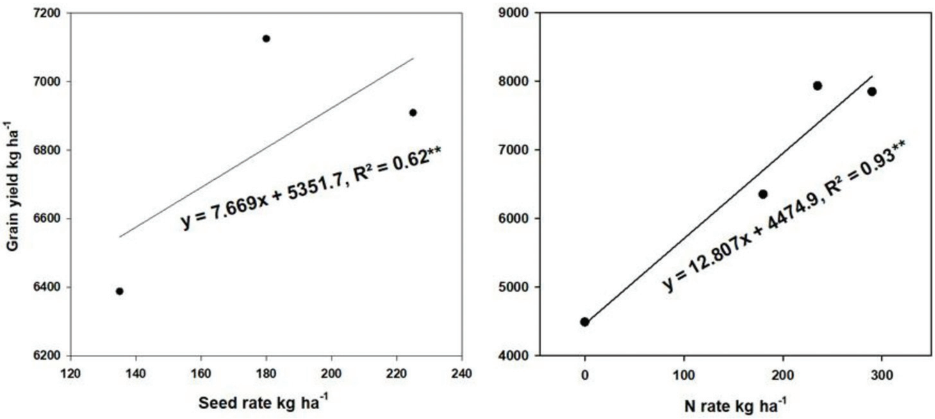

Figure 5. Estimating the regression line to compare N and seed rate for balancing grain yield and improving NU_E. * $p \leq 0.05$ and ** $p \leq 0.001$, respectively. The dots represent the mean value of grain yield under each seed rate (left) or nitrogen rate (right).

2.3. Accumulation, Translation, and Partitioning of DM

DM accumulation (DMA) was significantly affected by the rate of N application and SR (Table 2). Increasing the rate of application of N significantly increased DMA in all growth stages. The highest amount of DMA appeared during the jointing to the anthesis stage. SR significantly increased DMA at all growth stages. The significant effects of SR were up to 180 kg ha^{-1}, and beyond SR_{180} kg ha^{-1} was not further influenced (Table 2). Similarly, when N fertilizer increased beyond N_{235} kg ha^{-1}, the values of DMA were not significantly increased. At maturity, the highest increase in DM was found with $N_{235}SR_{180}$ treatment.

Table 2. Combination effects of N and SR on DM accumulation, translocation in 2018–2019.

Total DM Accumulation kg ha^{-1}						DM Translocation			
N kg ha^{-1}	**SR kg ha^{-1}**	**So-JT**	**JT-An**	**An-M**	**So-M**	**PTA kg ha^{-1}**	**CPT%**	**PAA kg ha^{-1}**	**CPA%**
N_0	SR_{135}	1959 ^{h}	6139 ^{b}	2680 ^{c}	10778 ^{g}	1214.1 ^{f}	30.3 ^{a}	4256 ^{d}	69.7 ^{e}
	SR_{180}	2314 ^{g}	6514 ^{b}	3250 ^{bc}	12079 ^{f}	1322.8 ^{f}	27.6 ^{b}	5072 ^{d}	72.4 ^{d}
	SR_{225}	2401 ^{f}	6682 ^{b}	3025 bc	12108 ^{f}	1439 ^{e}	29.7 ^{a}	5018 ^{d}	70.3 ^{e}
N_{180}	SR_{135}	2395 ^{f}	8585 ^{a}	3708 ^{b}	14689 ^{e}	1529.4 ^{de}	27.2 ^{bc}	6053 ^{c}	72.8 ^{cd}
	SR_{180}	2856 ^{b}	8904 ^{a}	4780 ^{a}	16539 ^{cd}	1633.8 ^{cd}	24.3 ^{d}	7276 ^{b}	75.7 ^{b}
	SR_{225}	2488 ^{e}	8587 ^{a}	4646 ^{a}	15721 ^{d}	1701.4 ^{c}	25.6 ^{cd}	7164 ^{b}	74.4 ^{bc}
N_{235}	SR_{135}	2668 ^{d}	9335 ^{a}	4731 ^{a}	16735 ^{c}	1918.7 ^{ab}	25.5 ^{cd}	7804 ^{ab}	74.5 ^{bc}
	SR_{180}	3019 ^{a}	10027 ^{a}	5130 ^{a}	18177 ^{ab}	1963.7 ^{a}	23.6 ^{de}	8727 ^{a}	76.4 ^{ab}
	SR_{225}	3033 ^{a}	9804 ^{a}	5064 ^{a}	17902 ^{ab}	2028.5 ^{a}	24.9 ^{d}	8490 ^{a}	75.1 ^{b}
N_{290}	SR_{135}	2719 ^{c}	9477 ^{a}	5048 ^{a}	17245 ^{bc}	1905.8 ^{ab}	24.4 ^{d}	8141 ^{ab}	75.6 ^{b}
	SR_{180}	2998 ^{a}	10066 ^{a}	5418 ^{a}	18483 ^{a}	1823.2 ^{b}	22.1 ^{e}	8776 ^{a}	77.9 ^{a}
	SR_{225}	3010 ^{a}	9649 ^{a}	5459 ^{a}	18119 ^{ab}	1906.7 ^{ab}	24 ^{de}	8518 ^{a}	76.4 ^{ab}
F-Value	N	1817 **	28.1 **	32.1 **	258 **	167.9 **	50.4 **	92.454 **	50.4 **
	SR	84 **	0.9	4.3 *	23.8 **	11.1 **	16 **	9.546 **	16 **
	N*SR	67.1 **	0.1	0.3	0.4	1.9	0.2	0.28	0.2

Note: S_O-JT, JT-An, An-M, S_O–M, PTA, CPT, PAA, and CPA represent sowing to jointing, jointing to anthesis, anthesis to maturity, sowing to maturity, pre-anthesis DM translocation amount, contribution of pre-anthesis translocation to grain, post-anthesis accumulation amount, and contribution of post-anthesis DM accumulation to grain, respectively. Different letters in the same column represent significant differences in mean values of three replicate plots according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

The translocation and contribution of DM were significantly affected by the application rate of N and SR (Table 2). With increasing N rate, pre-anthesis translocation (PTA), post-anthesis accumulation (PAA), and contribution of post-anthesis to grain (CPA) were significantly increased, while the contribution of pre-anthesis translocation to grain (CPT) was significantly decreased. The maximum value of PTA appeared in the N_{235} treatment, which was significantly higher than the N_{290} treatment. Furthermore, the value of PTA increased with increasing SR, while PAA and CPA significantly increased up to SR_{180} kg ha^{-1}. The CPT values first decreased and then increased as the SR increased.

The partition of DM into different parts of the plant differed between the N and SR treatments (Table 3). At the anthesis stage, the DM of culm + sheath was higher than the DM of rachis + glumes and the DM of rachis + glumes was higher than the DM of the leaves. However, at the harvesting stage, the grain DM was higher than the DM of the culm + sheath, the DM of culms + sheaths was higher than the DM of rachis + glumes, and the DM of the rachis + glumes was higher than the DM of leaves. The proportion of distribution of DM of grains, rachis + glumes, culms + sheathes, and leaves ranged from 37.1% to 45.8%, 12.7% to 14.1%, 33% to 41.4%, and 7.8% to 8.5%, respectively, at harvesting.

Table 3. Effects of N and SR on DM accumulation and partitioning at the anthesis and maturity stages in 2018–2019.

N kg ha^{-1}	SR kg ha^{-1}	Grain		Rachis + Glumes		Culms + Sheaths		Leaves	
		Anthesis	Maturity	Anthesis	Maturity	Anthesis	Maturity	Anthesis	Maturity
N_0	SR_{135}		4005 ^{g}	1576 ^{c}	1465 ^{f}	5442 ^{g}	4462 ^{c}	1080 ^{f}	846 ^{f}
	SR_{180}		4788 ^{f}	1821 ^{c}	1606 ^{e}	5631 ^{f}	4741 ^{bc}	1376 ^{e}	944 ^{e}
	SR_{225}		4851 ^{f}	1994 ^{c}	1606 ^{e}	5701 ^{f}	4682 ^{bc}	1389 ^{e}	968 ^{e}
N_{180}	SR_{135}		5623 ^{e}	2344 ^{bc}	1959 ^{d}	7217 ^{d}	5932 ^{a}	1420 ^{e}	1175 ^{d}
	SR_{180}		6712 ^{d}	2496 ^{bc}	2198 ^{bc}	7426 ^{c}	6299 ^{a}	1837 ^{c}	1330 ^{c}
	SR_{225}		6647 ^{d}	2517 ^{bc}	2218 ^{bc}	6716 ^{e}	5525 ^{ab}	1841 ^{c}	1330 ^{c}
N_{235}	SR_{135}		7535 ^{c}	3072 ^{ab}	2187 ^{c}	7156 ^{d}	5642 ^{a}	1775 ^{d}	1370 ^{b}
	SR_{180}		8324 ^{a}	3597 ^{a}	2366 ^{a}	7375 ^{c}	6017 ^{a}	2075 ^{a}	1468 ^{a}
	SR_{225}		8152 ^{ab}	3426 ^{ab}	2366 ^{a}	7341 ^{c}	5919 ^{a}	2070 ^{a}	1464 ^{a}
N_{290}	SR_{135}		7814 ^{bc}	3092 ^{ab}	2232 ^{b}	7199 ^{d}	5807 ^{a}	1906 ^{b}	1391 ^{b}
	SR_{180}		8244 ^{a}	3358 ^{ab}	2355 ^{a}	7641 ^{a}	6425 ^{a}	2066 ^{a}	1458 ^{a}
	SR_{225}		8087 ^{ab}	3058 ^{ab}	2337 ^{a}	7545 ^{b}	6241 ^{a}	2057 ^{a}	1453 ^{a}
F-Value	N		690 **	14.8 **	2002 **	2858.3 **	18.4 **	955.9 **	1001 **
	SR		60.1 **	0.92	207 **	96.3 **	2.3	324.9 **	85.5 **
	N*SR		2.64 *	0.17	6.29 **	61.7 **	0.58	11.1 **	2.92 *

Note: N and SR represent nitrogen application and seed rate, respectively. Different letters in the same column represent significant differences of mean values of three replicate plots at $p \leq 0.05$ levels according to Duncan′s test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

2.4. Accumulation, Translocation, and Partitioning of N

Accumulation of N (NA) at all parts of the plant was significantly increased by increasing N application and SR up to certain levels (N_{235}, SR_{180}) at both anthesis and maturity stages. At the maturity stage, the total content of N compared to control (N_0) treatment increased by 76.9%, 134%, and 139% in the treatments N_{180}, N_{235}, and N_{290}. The N content compared to SR_{135} was increased by 8.8% and 5% in the SR_{180} and SR_{225} treatments (Table 4). As well, N translocation before anthesis to grain (NTA), N accumulation after anthesis (NAA), and the contribution rate of NA after anthesis to grain (CAG) were significantly increased with the increase in N application. NTA increased 32.18% and 2.9% when N increased from N_{180} to N_{235} from N_{235} to N_{290}, respectively (average of three SR treatments). Furthermore, the contribution rate of pre-N translocation to grain (CTG) had the same trend as CPT. Furthermore, by increase in SR up to 180 kg ha^{-1}, NTA, NAA, and CAG were significantly increased, while CTG was at first significantly decreased then increased (Table 4).

Table 4. Combination effects of N and SR on total N accumulation and translocation in 2018–2019.

N kg ha^{-1}	SR kg ha^{-1}	Total N Accumulation kg ha^{-1} So-JT	JT-An	An -M	So-M	N Translocation NTA kg ha^{-1}	CTG%	NAA kg ha^{-1}	CAG%
N_0	SR_{135}	25.6 ^{h}	53.4 ^{i}	12.4 ^{g}	91.4 k	48.3 ^{j}	70.1 ^{ab}	27.4 j	29.9 ^{de}
	SR_{180}	28.5 ^{g}	60.4 ^{h}	14.5 ^{f}	103.4 ^{i}	55.5 ^{i}	69.4 ^{bc}	31.5 ^{h}	30.6 ^{cd}
	SR_{225}	28.8 ^{g}	60.4 ^{h}	11 ^{g}	100.2 ^{j}	55.3 ^{i}	71 ^{a}	29.2 ^{i}	29 ^{e}
N_{180}	SR_{135}	41.3 ^{f}	97.2 ^{g}	23.2 ^{e}	161.6 ^{h}	84.3 ^{h}	68.4 ^{cd}	48.9 ^{g}	31.6 ^{bc}
	SR_{180}	45.6 ^{e}	108.5 ^{e}	30.2 ^{cd}	184.3 ^{f}	97.8 ^{f}	68.2 ^{d}	56.5 ^{e}	31.8 ^{b}
	SR_{225}	46.1 ^{e}	100.6 ^{f}	29.1 ^{d}	175.8 ^{g}	94.7 ^{g}	68.3 ^{cd}	54.2 ^{f}	31.7 ^{bc}
N_{235}	SR_{135}	57.4 ^{d}	135.1 ^{d}	29.7 ^{cd}	222.2 ^{e}	118.1 ^{e}	67.5 ^{de}	70 ^{d}	32.5 ^{ab}
	$SR1_{80}$	61.7 ^{ab}	145.3 ^{a}	31.5 ^{c}	238.6 ^{ab}	125.9 ^{ab}	66.7 ^{e}	76.9 ^{a}	33.3 ^{a}
	SR_{225}	61.6 ^{ab}	136.3 ^{d}	31.5 ^{c}	229.4 ^{d}	122.1 ^{d}	67.3 ^{de}	72.7 ^{bc}	32.7 ^{ab}
N_{290}	SR_{135}	59 ^{c}	140.1 ^{b}	31.3 ^{c}	230.4 ^{d}	123.2 ^{cd}	67.8 ^{de}	72.1 ^{c}	32.2 ^{ab}
	SR_{180}	61.9 ^{a}	145.8 ^{a}	34.2 ^{b}	241.8 ^{a}	126.9 ^{a}	66.6 ^{e}	77.7 ^{a}	33.4 ^{a}
	SR_{225}	61 ^{b}	138 ^{c}	36.1 ^{a}	235 ^{c}	124.6 ^{bc}	67.4 ^{de}	74.2 ^{b}	32.6 ^{ab}
F-Value	N	1159 **	2229 **	628.1 **	6418 **	9584 **	44.2 **	436.6 **	44.2 **
	SR	270.4 **	377.9 **	31.8 **	1283 **	195.7 **	5.7 *	115.6 **	5.7 *
	N*SR	7.1 **	31.7 **	8.72 **	44.3 **	15.81 **	1	3.03 *	1

Note: SO-JT, JT-An, An-M, SO–M, NTA, CTG, NAA, and CAG represent jointing, jointing to anthesis, anthesis to maturity, sowing to maturity, pre-anthesis N translocation, contribution rate of N translocation to grain, nitrogen accumulation amount, and contribution rate of N accumulation to grain, respectively. Different letters in the same column represent significant differences in mean values of three replicate plots according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

Plant N partitioning was also influenced by N and SR. Compared with the control treatment (N_0), the N_{180}, N_{235}, and N_{290} treatments increased the N content by 53.8%, 153%, and 144% in the rachis + glumes, by 84.2%, 149.2%, and 157.6% in the culms + sheathes, and by 65.8%, 107.7%, and 109.4% in the part of leaves, respectively, at anthesis stage. However, this increase in N at the harvesting stage was by 78.7%, 140.3%, and 145.6% for grains, by 52.9%, 98.5%, and 103% for rachis + glumes, by 61.5%, 88.1%, and 99% for culms + sheathes, and by 113.7%, 182%, and 186% for leaves in treatments N_{180}, N_{235}, and N_{290} compared to N0 treatment, respectively. Additionally, compared to the SR_{135} treatment, the SR_{180} and SR_{225} treatments increased N content by 8.6% and 1% at the rachis + glumes, by 1.7% and 2% at the culms + sheathes, and by 16% and 3.7% at the parts of leaves, respectively, at anthesis stage. Compared to SR_{135} treatment, the N content of the SR_{180} and SR_{225} treatments increased by 9.8% and 6.2% in grains, by 5.5% and 1.8% in the rachis + glumes, by 5.1% and −2% in the culms + sheathes, and by 5.5% and 2.7% in the leaves, respectively, at the maturity stage (Table 5). The range of the N distribution ratio of different parts, i.e., rachis + glumes, culms + sheathes, and leaves were from 17% to 21.9%, from 39.1% to 47.1%, and from 33.5% to 41.6% at anthesis, respectively. The maturity stage range of N distribution range was 75.4% to 79.2%, from 5.8% to 7.4%, 8.8% to 11.9%, and from 5% to 6.4% for grains, rachis + glumes, culms + sheathes, and leaves, respectively (Table 5).

Table 5. Combination effects of N and SR on N partitioning during anthesis and maturity stages in 2018–2019.

N kg ha^{-1}	SR kg ha^{-1}	N at Grain		N at Rachis + Glumes		NA at Culms + Sheaths		N at Leaves	
		Anthesis	Maturity	Anthesis	Maturity	Anthesis	Maturity	Anthesis	Maturity
N_0	SR_{135}		68.9 ^{f}	15 ^{g}	6.8 ^{c}	34.9 ^{g}	10.9 ^{e}	29.2 ^{i}	4.9 ^{d}
	SR_{180}		80 ^{e}	17.1 ^{f}	7.1 ^{c}	35.1 ^{g}	11.2 ^{e}	36.8 ^{g}	5.2 ^{d}
	SR_{225}		77.8 ^{ef}	18.2 ^{f}	6.6 ^{c}	34.9 ^{g}	10.6 ^{e}	36.1 ^{h}	5.1 ^{d}
N_{180}	SR_{135}		123.2 ^{d}	25.7 ^{e}	10 ^{b}	65.2 ^{e}	18.1 ^{cd}	47.6 ^{f}	10.3 ^{c}
	SR_{180}		143.4 ^{c}	26.2 ^{e}	10.8 ^{b}	67.1 ^{d}	18.8 ^{bc}	60.8 ^{e}	11.3 ^{b}
	SR_{225}		138.5 ^{c}	25.1 ^{e}	10.3 ^{b}	60.6 ^{f}	15.9 ^{d}	60.9 ^{e}	11 ^{bc}
N_{235}	SR_{135}		174.9 ^{b}	40.3 ^{c}	13.2 ^{a}	87.7 ^{b}	20.1 ^{abc}	64.5 ^{d}	14 ^{a}
	SR_{180}		188.9 ^{a}	45.4 ^{a}	13.9 ^{a}	88.4 ^{b}	21 ^{abc}	73.3 ^{a}	14.7 ^{a}
	SR_{225}		181.4 ^{ab}	41.2 ^{c}	13.4 ^{a}	85.7 ^{c}	20.2 ^{abc}	70.9 ^{b}	14.4 ^{a}

Table 5. *Cont.*

N kg ha^{-1}	SR kg ha^{-1}	N at Grain		N at Rachis + Glumes		NA at Culms + Sheaths		N at Leaves	
		Anthesis	Maturity	Anthesis	Maturity	Anthesis	Maturity	Anthesis	Maturity
N_{290}	SR_{135}		181.7 ^{ab}	40.8 ^{c}	13.5 ^{a}	89.1 ^{b}	20.7 ^{abc}	69.2 ^{c}	14.4 ^{a}
	SR_{180}		190.4 ^{a}	43.5 ^{b}	14.1 ^{a}	91.2 ^{a}	22.5 ^{a}	73 ^{a}	14.8 ^{a}
	SR_{225}		184.9 ^{a}	38.1 ^{d}	13.9 ^{a}	89.5 ^{ab}	21.7 ^{ab}	71.4 ^{b}	14.5 ^{a}
F-Value	N		845.1 **	2010.4 **	322.7 **	5132.3 **	83.3 **	24379 **	595 **
	SR		19.80 **	36.8 **	4.35 *	19.9 **	2.02	2256 **	3.63 *
	N*SR		0.98	13.9 **	0.2	5.6 *	0.66	172.9 **	0.3

Note: N, SR, and NA represent nitrogen application, seed rate, and N accumulation, respectively. Different letters in the same column represent significant differences in mean values of three replicate plots according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively. Revision as above.

2.5. N Use Efficiency (NU_E) Parameters

N rates, SR, and their interaction had a significant effect on N agronomy efficiency (NA_E), N uptake efficiency (NUP_E), and N partial factor productivity (NPFP). Increasing N level up to N_{235} kg ha^{-1}, NA_E, NUP_E, N recovery efficiency (NR_E), and N harvest index (NHI) were significantly increased. However, increasing N levels beyond N_{235} kg ha^{-1}, NA_E, NR_E, and NUP_E were significantly decreased. The NPFP values were decreased at all N levels. Furthermore, the NA_E, NR_E, NUP_E, NPFP, and NHI values decreased by 12.8%, 10.4%, 17.3%, 13.6%, and 0.4%, respectively, at N_{290} treatment compared to N_{235} treatment. Similarly, SR had a considerable effect on the NU_E parameters, but the effect of SR was less compared to the N treatment. Maximum values for the parameters NA_E, NR_E, and NUP_E were observed from the combination of treatment with N_{235} and SR_{180} (Table 6).

Table 6. Combination effects of N and SR on N use efficiency parameters in 2018–2019.

N Rate kg ha^{-1}	SR kg ha^{-1}	NA_E	NR_E	NUP_E	NPFP	NHI
		kg kg^{-1}	%	kg kg^{-1}	kg kg^{-1}	%
N_0	SR_{135}					0.75 ^{f}
	SR_{180}					0.77 ^{de}
	SR_{225}					0.77 ^{de}
N_{180}	SR_{135}	9.0 ^{f}	39 ^{e}	0.9 ^{c}	31.2 ^{c}	0.76 ^{ef}
	SR_{180}	10.7 ^{de}	44.9 ^{cd}	1.02 ^{a}	37.3 ^{a}	0.78 ^{bc}
	SR_{225}	10 ^{e}	42 ^{de}	0.98 ^{a}	36.9 ^{a}	0.79 ^{ab}
N_{235}	SR_{135}	14.4 ^{a}	53.4 ^{a}	0.95 ^{b}	30.8 ^{c}	0.79 ^{ab}
	SR_{180}	14.4 ^{a}	55.2 ^{a}	1.02 ^{a}	34 ^{b}	0.79 ^{ab}
	SR_{225}	13.5 ^{b}	52.7 ^{a}	0.98 ^{b}	33.3 ^{b}	0.8 ^{a}
N_{290}	SR_{135}	13.4 ^{b}	48.8 ^{b}	0.8 ^{d}	27.4 ^{e}	0.79 ^{ab}
	SR_{180}	12.1 ^{c}	48.6 ^{b}	0.83 ^{d}	28.9 ^{d}	0.79 ^{ab}
	SR_{225}	11.4 ^{cd}	47.3 ^{bc}	0.81 ^{d}	28.4 ^{de}	0.79 ^{ab}
F-value	N	156.4 **	82.6 **	3774.8 **	180.8 **	17.6 **
	SR	6.4 **	4.4 *	20.04 **	55.4 **	7.1 **
	N*SR	8.76 **	2.16	4.27 **	9.12 **	1.88

Note: N, SR, NA_E, NR_E, NUP_E, NPFP, and NHI represent nitrogen rate, seed rate, nitrogen agronomy efficiency, N recovery efficiency, N uptake efficiency, N partial factor productivity, and N harvest index, respectively. Different letters in the same column represent significant differences in mean values of three replicate plots according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

2.6. Correlation Analysis

2.6.1. Correlation of GY with Agronomic and Photosynthesis Traits

The key relationships between the GY-related parameter variables are shown in Table 7. There was a significant positive correlation between GY and NS, NGS, TGW, PH, HI, Pn, Gs, SPAD value, and LAI. However, there was no significant relationship between GY and PH (Table 7).

Table 7. Correlation analysis of grain yield with agronomic and photosynthesis traits.

	GY	NS	NGS	TGW	PH	HI	Pn	Gs	SPAD	LAI
GY	**1**	0.854 **	0.893 **	0.626 **	0.116	0.879 **	0.917 **	0.788 **	0.889 **	0.961 **
NS		1	0.535 **	0.418 *	0.346*	0.733 **	0.641 **	0.492 **	0.638 **	0.777 **
NGS			1	0.613 **	−0.08	0.804 **	0.942 **	0.860 **	0.909 **	0.900 **
TGW				1	0.212	0.432 **	0.676 **	0.566 **	0.699 **	0.633 **
PH					1	−0.102	0.031	−0.138	0.22	0.117
HI						1	0.747 **	0.660 **	0.673 **	0.821 **
Pn							1	0.852 **	0.951 **	0.923 **
Gs								1	0.813 **	0.814 **
SPAD									1	0.894 **
LAI										1

Note: GY: grain yield; NS: number of spikes; NGS: number of grains per spike; TGW: 1000 grain weight per gram; PH: plant height (cm); HI: harvest index; Pn: net photosynthesis; Gs: stomatal conductance; SPAD: leaf greenness; LAI: leaf area index. * and ** mean significantly correlation at * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

2.6.2. Relationship between GY and Enzyme Activities and NUE Parameters

Grain yield had a significant and positive relationship with nitrogen reductase (NR) and glutamine synthesis (GS) enzymes, N agronomic efficiency (NA_E), N recovery efficiency (NR_E), and N uptake efficiency (NUP_E). However, GY had a significantly negative relationship with N translocation's contribution before anthesis to grain (CTG) (Figure 6).

Increasing N application from 235 to 290 kg ha^{-1}, GY did not increase significantly (0.4%) while NA_E, NR_E, and NUP_E were decreased 12.8%, 10.4%, and 17.3%, respectively. Maximum GY and highest values of NU_E parameters, particularly NA_E, NR_E, and NUP_E, were observed from the combination of treatment with N_{235} and SR_{180} (Tables 1 and 6).

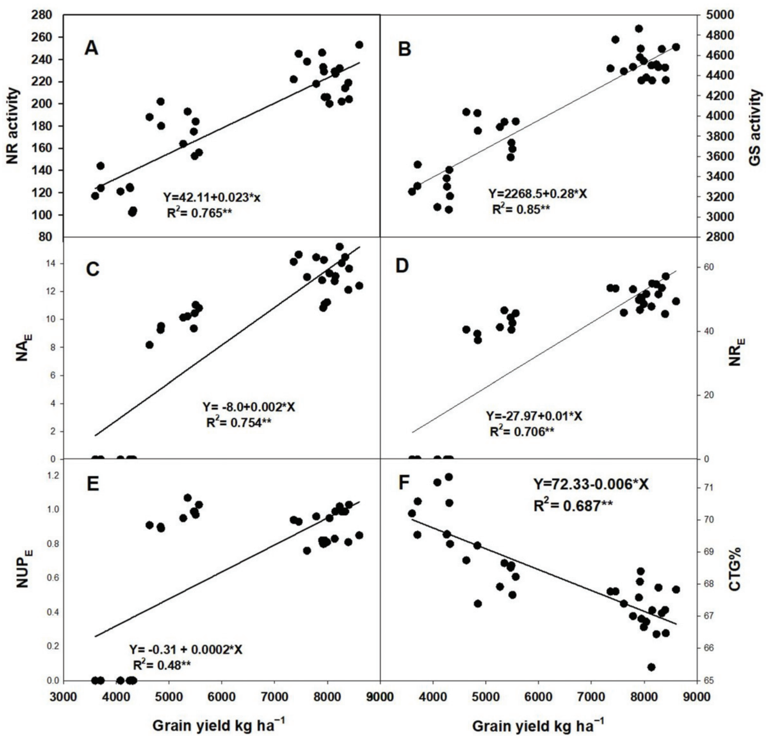

Figure 6. Regression analyses among GY and nitrate reductase (NR)/(**A**) glutamine synthesis (GS)/(P**B**), N agronomy efficiency (NAE)/(**C**), N recovery efficiency (NR_E)/(**D**), N uptake efficiency (NUP_E)/(**E**), and contribution of N translocation to the grain after the anthesis stage (CTG)/(**F**), respectively. * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

3. Discussion

DM and N translocation are well-known to greatly contribute to the final GY [22,23]. However, there is a lack of research on the combined effects of N and SR on the DM and N translocation, NU_E parameters, growth physiological parameters, and their relationship with GY in winter wheat. The effects of the excessive rate of N and N′s compensation by increasing SR on final GY and NU_E parameters were unknown.

3.1. Physiological Characteristics

The plant leaf′s photosynthesis capacity plays a crucial role in plant growth and grain yield [24], and approximately 70% of productivity is derived from post-anthesis photosynthesis. In the present study, increasing N application and decreasing SR, resulted in a significantly increased Pn, Gs, and SPAD values in both growing seasons. Furthermore, LAI was significantly increased by increasing N application and SR (Figures 1–3). However, it has shown that the maximum values for Pn, Gs, and SPAD parameters were exhibited from the combination of N_{290} + SR_{135} treatment but there were no significant differences between the mentioned and N_{235} + SR_{180} treatment. The main reason for decreasing the Pn, Gs, and SPAD values by increasing SR might be due to more competition and the overcrowded shading effect as reported by [25]. In addition, N reductase (NR) and glutamine synthesize (GS) increased significantly with the N application rate and the declaration of SR. Notably, there was no significant difference for the value of both mentioned enzyme activities when N fertilizer amount increased from N_{235} to N_{290}. These findings suggest N and SR′s optimization benefits for maintaining strong photosynthesis capacity and N assimilation ability in wheat plants.

3.2. Grain Yield (GY) and N Use Efficiency (NU_E)

Simultaneous improvement in GY and NU_E of wheat is an important objective in modern agriculture management. Here, we investigated the suitable combinations of N and SR to obtain higher GY and NUE. The maximum value was observed with the treatment of N_{235} + SR_{180} kg ha^{-1} in both growing seasons. The previous finding can explain that too high plant density had no significant effect on wheat grain yield [11,26]. According to our results, yield loss caused by reducing the N rate can be compensated by increasing SR (Figure 2). It was estimated by a linear regression that every decrement of 1 kg N ha^{-1} can be replaced by adding 0.6 kg ha^{-1} SR. The GY obtained by adding SR is clearly attributed to the increasing spike number (SN) (Table 1). Moreover, the yield in 2017–2018 (Y1) was much lower than that in 2018–2019 (Y2), which may be due to the adverse weather conditions. There was excessive rainfall and less sunshine during grain filling in the first growing season (Figure 1), leading to lower yield components and GY [1].

NU_E results from the incorporation of N-uptake efficiency (NUP_E) and N-utilization efficiency (NUT_E) [27]. In detail, NUP_E the plant′s capacity to extract N from the soil, and depending on the root structure and the relation of N transporters [28]. In this study, increasing N application from N_{235} to N_{290} resulted in a decrease in NAE, NR_E, NUP_E, and NPFP by 12.8%, 10.4%, 17.3%, and 13.6%, respectively. Similarly, a result was obtained by [29] that treatment with N_{240} and N_{300} compared to treatment with N180, NPFP were decreased by 24.5% and 37.4% and NA_E were decreased by 23.5% and 31.9%, respectively. The decrease in NU_E after the optimal rate might be due to more losses by increasing N application according to the previous finding of [11]. Here, our results further confirmed that NU_E components were significantly increased up to a certain amount of SR (180 kg ha^{-1}), which is in good agreement with the previous study [30]. This increase in the NU_E response to the high SR could be due to an increase in the density of the roots in the soil, which enhanced N from deeper parts of the soil. Therefore, it is not surprising that maximum values of NA_E, NR_E, and NUP_E were also observed from the optimal combined treatment (N_{235} + SR_{180}), which was also the case for grain yield (Table 2). Our study thereby provides a practical management method approaching higher GY and NUP_E by the optimization of N and SR.

3.3. Accumulation and Translocation of DM and N

Total dry matter accumulation and partitioning into separate parts of the plant were significantly affected by combined N and SR′s combined treatments. The maximum value of total dry matter and individual parts especially grains, rachis + glumes, and leaves, was also obtained from the combination of N_{235} + SR_{180} treatment. Recent studies reported that the contribution of pre-anthesis translocation to grain was significantly decreased when the N application rate increased [31,32]. Similarly, the result of the current experiment showed that the contribution of pre-anthesis DM translocation was significantly decreased with increasing N rate, while pre-anthesis DM translocation, post-anthesis DM accumulation, and contribution of post-anthesis DM accumulation to grain were significantly increased. The main reason for the decreasing contribution of pre-anthesis DM translocation to grain might be that early senescence occurred due to N deficiency, which would speed up the pre-translocation from leaf and stem to spike. Furthermore, increasing seed rate significantly increased pre-anthesis dry matter translocation, post-anthesis DM accumulation, and contribution of post-anthesis DM accumulation to the grain. Parallel to our finding, it was reported that post-heading DM and N accumulation was significantly increased with increasing SR [33]. It should be noted that an excessive amount of N application (N_{290} kg ha^{-1}) as well as SR (SR_{225} kg ha^{-1}) did not increase the amount of DM translocation.

Total N accumulation, partitioning, and translocation showed the same trend with the above part of DM. Both N and SR up to optimal levels (N_{235}, SR_{180}) significantly increased the total N content of individual parts. The same finding reported that no further increase was observed in the uptake of N at N fertilizer and the density of the plant beyond 240 kg N ha^{-1} and 405 plants m^{-2} [21,34]. N translocation, postanthesis N accumulation, and postanthesis N accumulation contribution of post-anthesis N accumulation to the grain were higher in the high N treatments compared to control and low N treatment. A similar result was found that N translocation and post-anthesis N accumulation were enhanced with increasing N application rate [23]. In the current experiment, N translocation, N accumulation after anthesis, and contribution of post-anthesis to grain response to seed rate were significantly increased from SR $_{135}$ to SR $_{180}$ kg ha^{-1}.

3.4. Relationship of GY with Related Parameters

Our results showed that GY has a significant and positive correlation with Pn, Gs, SPAD value, LAI, and other GY components (Table 7). This is similar to the results from the study by Jiang et al. [24]. Furthermore, the regression analyses also revealed that GY had a positive correlation with NR, GS, NA_E, NR_E, and NUP_E while showing a negative correlation with CTG (Figure 6). It was also observed that NR and GS activities were highly positively correlated with photosynthesis capacity, which is consistent with previous studies [35,36]. Here, we found that GY had a significant positive correlation with NU_E parameters such as NA_E, NRE, and NUP_E. This was not in agreement with the previous finding that GY showed a negative correlation with NU_E [37,38]. The reason might be due to a certain amount of N + SR (N_{235} + SR_{180}), in which both NU_E and GY were significantly higher up to the previous partnership. In conclusion, we found that N and SR′s improper rate cannot increase GY, but significantly decreased NU_E. In this regard, to achieve the maximum GY and NU_E, it would be better to use the optimal amount of both N and SR, which is the result of the current experiment, and the suitable combined treatment was N_{235} + SR_{180}. Furthermore, by using a suitable combination of N and SR (SR_{180} N_{235}), replacing N to SR especially for balancing GY and NU_E would be the best method for sustainable agriculture. According to our findings, we infer that, based on low SR (SR_{135}), 1 kg ha^{-1} N could be saved by increasing approximately 0.6 kg ha^{-1} SR.

4. Materials and Methods

4.1. Plant Material and Experimental Site

The field experiment was conducted during two successive growing seasons (2017–18 and 2018–19) at the XuYi Rice and Wheat demonstration center (118°43′ N and latitude 32°59′ E) in Jiangsu province, China. The winter wheat cultivar Ningmai 13 was used in both growing seasons. The soil type was clay loam and the pH was 6.8. It contained 31.07 g kg^{-1} of organic matter, 2449 g kg^{-1} of available N, 27.3 mg kg^{-1} of available phosphate, and 240 mg kg^{-1} of available potassium. Seeds were sown on 31 October 2017, and 1 November 2018, and the crop was harvested on 3 June 2018, and 6 June 2019, respectively.

4.2. Experiment Design

The experiment was carried out according to the split-plot design with three replicates. The main plot consisted of three seed rates (SR_{135}, SR_{180}, and SR_{225} kg ha^{-1}) and the subplot comprised two levels of doses of N in the first year (N_{235} and N_{290} kg ha^{-1}) and three N levels in second year of the experiment (N_{180}, N_{235}, and N_{290} kg ha^{-1}). At the first growing season, there was no significant effect between N_{235} and N_{290} for GY, because of that we added N_{180} kg ha^{-1} treatment at the second growing season to determine the N effect as well to determine the optimum rate of N for GY. An N-control plot (N_0) was also used at the second growing season per replication for the calculation of NU_E parameters. N fertilizer was applied as urea (46%), phosphorus (P) and potassium (K) fertilizer as calcium superphosphate (15%), and potassium chloride (60%) at the rates of 120 (P_2O_5) and 120 (K_2O) kg ha^{-1}. All of the phosphorus and potassium fertilizer and 70% of the total amount of N fertilizer were spread by hand before plowing at the time of sowing. The remaining N fertilizer (30%) was applied at the first node (31) according to BBCH. Each plot size was 4 × 3 m and consisted of 12 rows with a row-to-row distance of 25 cm.

In this experiment, the rice–wheat rotation system was undertaken for the long term. Rice cultivation techniques such as puddling, transplanting, and flooding, and the whole amount of straw returned to the field almost in the last decade. Only 5 cm of rice straw remains above the ground. Moldboard plough was followed by rotary plough as primary and secondly tillage. The depth of moldboard tillage was 20 cm and that of the rotary plough was 10 cm. Wheat seeds were sown with a seed drill precise machine with surface stubble plowing and roll compaction. For high-yield production, insects, diseases, and weeds were controlled two times by spraying insecticide (Biscaya), fungicide (Capalo), and herbicide (sulfosulfuron) during both growing seasons.

4.3. Grain Yield and Yield Components

Uniform plants at the flowering stage were tagged with labels and were sampled at a later stage. At the maturity stage, ears/spikes f rom the area of 0.5 m^2 (without taking a sample) of each plot were collected to determine GY and yield components.

4.4. Photosynthesis, SPAD, LAI, N, and Enzyme Activities in Leaves

Photosynthesis (Pn), stomatal conductance (Gs), SPAD, and LAI were measured at the jointing stage, anthesis, 10 days after anthesis (DAA), and 20 DAA. Pn and GS were measured by a portable gas exchange analyzer (LI-6400XT;LI-COR-Inc., Lincoln NE, USA) at 9:00–11:00 a.m. on a sunny day. The concentration of CO_2 in the leaf chamber, light intensity, and relative humidity were set as 380 μmol mol^{-1}, 1000–1100 μmol $m^{-2}s^{-1}$, and 500 mL min $^{-1}$, respectively. The SPAD value was determined by Minolta 502 chlorophyll meter (Minolta, Japan). LAI was measured with using a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA). N concentration was determined by the micro Kjeldahl method [39]. Nitrate reductase (NR) and glutamine synthesis (GS) were determined according to the method previously described by [36] and [40].

4.5. Dry Matter (DM) and N Translocation

DM and N accumulation (DMA and NA), translocation, and their contribution were estimated according to [31] and [41] by using the following equations (Table 8).

Table 8. Equations for estimating dry matter and nitrogen translocation.

Parameters		**Equation**	**Unit**
Abbreviation	**Denotation**		
PTA	Pre-anthesis DM translocation	DM of vegetative parts at anthesis—at maturity	kg ha^{-1}
CPT	Contribution of pre-anthesis DM translocation to grain	PTA ÷ GY at maturity × 100	%
PAA	Post-anthesis DM accumulation	Biomass at maturity- biomass at anthesis	kg ha^{-1}
CPA	Contribution of post-anthesis DM accumulation to grain	PAA ÷ GY at maturity × 100	%
NTA	Pre-anthesis N translocation	N of vegetative parts at anthesis—at maturity	kg ha^{-1}
CTG	Contribution of pre-anthesis N translocation to grain	NTA ÷ grain N×100	%
NAA	Post-anthesis N accumulation	Plant N accumulation at maturity—N accumulation at anthesis	kg ha^{-1}
CAG	Contribution rate of post-anthesis N accumulation to grain	NAA ÷ grain N ×100	%

4.6. Use Efficiency (NU_E) Parameters

NU_E parameters were determined using the following equations described by [17], and [42] (Table 9).

Table 9. Equations for estimating NU_E parameters.

Parameters		**Equation**	**Unit**
Abbreviation	**Denotation**		
NA_E	N agronomy efficiency	(GY with N—GY without N) ÷ N application rate	kg kg^{-1}
NR_E	N recovery efficiency	(total N uptake with N- total N uptake without N) ÷ N application rate	%
NUP_E	N uptake efficiency	Above-ground N at harvesting ÷ N application rate	%
NPFP	N partial factor productivity	GY ÷ N application rate	kg kg^{-1}
NHI	N harvest index	Grain N accumulation at maturity/plant N accumulation at maturity	mg mg^{-1}

4.7. Weather Condition

Monthly average temperature, rainfall, and sunshine at the experimental site over two successive years (2017–2018 and 2018–2019) are presented in Figure 7. There was considerable variation between the two growing seasons. At the active tiller stage (from late January to the end of the first week of February), the minimum temperature in 2017–2018 was lower (−4.5 °C) compared to that of the second growing season (−0.5 °C). At the anthesis stage, the average rainfall in the first growing season was 538 mm, which was 64.02% higher than that in the second growing season (328 mm).

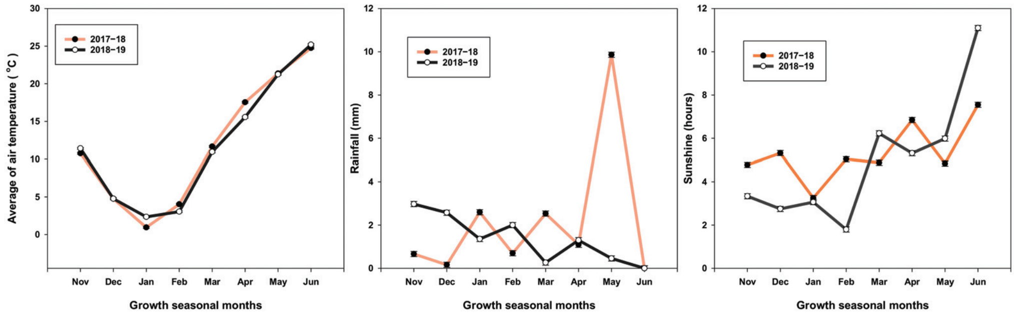

Figure 7. Metrological data: (monthly average temperature, rainfall (mm), and sunshine per hour) in two successive growing season.

4.8. Statistical Analysis

Two-way ANOVA (SPSS version 17.1) was used for analyzing the variance among different treatments. The means were tested with the least significant difference at the 0.05 probability level ($p \leq 0.05$ by Duncan's). Pearson's correlation between grain yield and related parameters were calculated through the SPSS version17.1. All graphs and linear regression analyses was done by sigmaplot 14.0 software (Chicago, IL, USA).

5. Conclusions

In summary, the GY, DMA, NAC, NU_E parameters and physiological parameters increased significantly with the combination of N_{235} and SR_{180}. However, the excessive rate of N application cannot increase GY and other parts of plant DM but it decreased NA_E, NR_E, and NUP_E. Our result confirmed that maximum GY and higher NU_E components could be achieved via avoiding excessive use of N, and optimizing the compensation effect of increasing SR for reducing N application.

Author Contributions: M.H., Q.Z. and D.J. designed the experiments. M.H. analysed data, interpreted data, and wrote the original draft of the manuscript. M.H., A.S. and M.S.J. carried out the experiments, curated and analyzed data. M.H., J.C., X.W., Q.Z. and T.D. were involved in the management of the experiment. D.J. participated in supervision of the project. M.S.J., Q.Z. and D.J. participated in the critical reading and discussion of the manuscript, H.M. did formal analysis and methodology. All authors have read and agreed to the published version of the manuscript.

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plants

Article

Agro-Physiological Traits of Kaffir Lime in Response to Pruning and Nitrogen Fertilizer under Mild Shading

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Abstract: Mild shading has been reported to increase leaf production in kaffir lime (*Citrus hystrix*) through the improvement of agro-physiological variables, such as growth, photosynthesis, and water-use efficiency; however, there is still a knowledge gap concerning its growth and yield after experiencing severe pruning in harvest season. Additionally, a specific nitrogen (N) recommendation for leaf-oriented kaffir lime is still unavailable due to its lesser popularity compared to fruit-oriented citrus. The present study determined the best pruning level and N dose based on agronomy and the physiology of kaffir lime under mild shading. Nine-month-old kaffir lime seedlings grafted to rangpur lime (*C. limonia*) were arranged in a split-plot design, i.e., N dose as a main plot and pruning as a subplot. Comparative analysis resulted in 20% higher growth and a 22% higher yield in the high-pruned plants by leaving 30 cm of main stem above the ground rather than short ones with a 10 cm main stem. Both correlation and regression analysis strongly highlighted the importance of N for leaf numbers. Plants treated with 0 and 10 g N plant^{-1} experienced severe leaf chlorosis due to N deficiency, while those treated with 20 and 40 g N plant^{-1} showed N sufficiency; thus, the efficient recommendation for kaffir lime leaf production is 20 g N plant^{-1}.

Keywords: *Citrus hystrix*; chlorosis; leaf production; photosynthesis; shading

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1. Introduction

Citrus is one of the leading, popular horticultural fruit commodities commonly used for fresh food and beverages [1–3]. Published biogeographic, genomic, and phylogenetic analyses determine the Southeast Himalayan foothills as the center of origin for most citrus species [4]. As one of the interesting *Citrus* taxa, lime is reported to have highly polymorphic characteristics, derived from four major *Citrus*, namely *C. medica*, *C. maxima*, *C. micrantha* and *C. reticulata*) [5]. In contrast, the kaffir lime is classified as a relatively minor citrus and apparently wild, native to central Malesia or the Southeast Asian region [6]. This lime is well-designed as a leaf-oriented target due to its aromatic leaves being used as spices in numerous Asian dishes [7–9]. Aside from its fragrance, the bifoliate characteristics of kaffir lime leaves can be used to differentiate this species from others [10,11]. The leaf of the kaffir lime is also famous for producing essential oils [12] that possess several bioactivities, such as antifeedant [13], antibacterial [14], and larvicide [15]. Due to its importance as a spice and an essential oil raw material, the demand for kaffir lime leaves is potentially increased. Thus, some effort is required to meet those needs. General strategies to boost plant production could employ both input intensification and land expansion [16].

Kaffir lime generally grows naturally or is planted in polyculture in the yard by residents with a non-intensive cultivation system [7,9]. In nature, kaffir lime typically grows from seed and shows a high tree appearance, long and large thorns, dense canopy and branches, so leaf harvest is less effective and efficient. On the other hand, several local communities in Tulungagung, Indonesia have implemented monoculture and semi-intensive kaffir lime cultivation [9]. Previous research has also proven that modification of cultivation under low-level shading (light reduction of about 23%) can produce beneficial stress, increasing the growth rate and yield of kaffir lime leaves by 84 and 63%, respectively [17]. Although that increasing response has been reported in the first harvest period, further research is urgently needed to confirm yields in the following period.

Kaffir lime experiences heavy pruning during its harvesting season. Heavy pruning is carried out by cutting most of the canopy and leaving a portion of the main stem for successive growth in the next period [9]. The remaining part of the main stem should be studied further since no standard has been set. Based on the results of interviews with several farmers, the previous studies reported a variation in the height of the postharvest main stem of between 10 and 30 cm above ground level [9]. The difference in that height is associated with the severity level of pruning. The height of the remaining main stem should be studied further as it relates to food reserves for growth in further seasons. Pruned plants may likely experience a different source and sink balance condition than unpruned ones. Too severe pruning may result in improper vegetative growth. Earlier studies reported a vegetative growth reduction of mandarin citrus due to heavy pruning [18]. Concerning leaf-oriented citrus such as kaffir lime, vegetative growth inhibition can directly threaten yields and profits. Therefore, there is an urgency to obtain the best pruning level for kaffir lime.

In addition to the importance of determining the pruning severity, optimizing leaf production in kaffir lime agribusiness needs to be supported by the best nitrogen (N) fertilizer doses. N is the imperative macronutrient for normal plant growth and development [19]. More specifically, this nutrient is required for producing chlorophyll, proteins, nucleic acids [20], amino acids, and sugar [21]. Thus, plant productivity highly depends upon N fertilization [22,23]. Previous studies have proven the role of N in increasing the vegetative growth of Eureka lemons and Maltese sweet citrus [24]. Concerning the kaffir lime, previous studies reported a strong and positive correlation between leaf nitrogen status and leaf oil production [25], strengthening the argument that N is urgently needed to produce good quality kaffir lime leaves. Unfortunately, there is no specific N-fertilizer dosage recommendation for leaf-oriented citrus such as kaffir lime. In Indonesia, the citrus research center has issued a recommendation only for fruit-oriented citrus, with a range of 10–20 g N per plant, for 1-year-old plants [26]. This dosage can be used as a reference for compiling specific recommendations for leaf-oriented kaffir lime cases.

Interestingly, there are some knowledge gaps pertaining to kaffir lime leaf production, i.e., the confusion resulting from a variation in the height of the postharvest main stem, the lack of information on the growth and yield in the following post-pruning period under similar mild-shading conditions, and the missing specific N recommendations for leaf-oriented production. Therefore, the present study aimed to determine the best pruning level and N dose based on the agro-physiological characteristics of kaffir lime under mild shading.

2. Results

2.1. Nitrogen Status in Soil, Leaves, and Canopy N Uptake

The present experiment measured both N status in leaves and soil at 90 DAT, and the results showed no significant differences in total soil N levels under different doses of N fertilizer applied (Figure 1A). The total N content of the soil in all the treatments ranged from 0.19 to 0.20%. In contrast, N-fertilizer doses had a noticeable effect on the total N content of the leaf tissue, with the highest N–leaf tissue in the highest N-fertilizer dose (40 g plant^{-1}), while the lowest N–leaf tissue in control/no N fertilizer applied. Compared to the control, the increase in N–leaf tissue was varied, by about 13% on 10 g N plant^{-1}, 17% on 20 g N plant^{-1}, and 34% on 40 g N plant^{-1}. Similarly, the increase in N-fertilizer doses was surely followed by a significant improvement in canopy N uptake (Figure 1B).

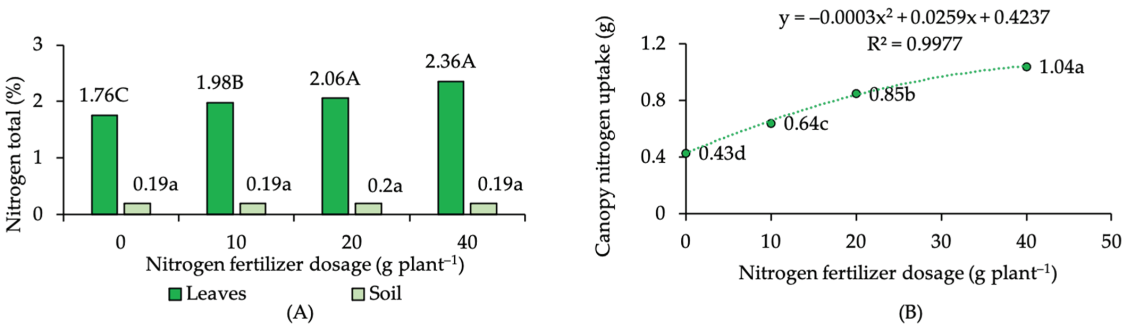

Figure 1. (**A**) Bar chart of nitrogen status on kaffir lime leaves and soil, and (**B**) regression curve of kaffir lime canopy nitrogen uptake under different nitrogen-fertilizer dosage. Note: mean values followed by the same letter are not significantly different based on DMRT at α 0.05.

The results of the regression analysis showed a high coefficient of determination (R^2) of 0.99. It was likely that the N-fertilizer dose variable could be used to estimate kaffir lime canopy N uptake by employing the provided mathematical equation. Additionally, correlation analysis also confirmed (i) the insignificant and weak correlation between N–soil and N–leaf tissue; and (ii) the significant, positive, and strong correlation between N–leaf tissue and canopy N uptake (r 0.97) (Table 1). The importance of N, as represented by canopy N uptake, for plant growth and physiological processes was also proved by correlation analysis, since canopy N uptake showed significant, positive, and strong correlation to certain variables of plant growth and physiology, namely relative growth rate, shoot number, leaf numbers, plant fresh weight, and leaf chlorophyll content.

Table 1. Pearson correlation analysis between nitrogen status, characteristics of production and physiology of kaffir lime.

	NTS	NLC	CNU	RGR	PH	SN	LN	LFW	PFW	PR	SC	TR	CA	CB	CT
NLC	0.05														
CNU	0.28	0.97													
RGR	0.42	0.93	0.98												
PH	0.57	0.84	0.94	0.97											
SN	0.15	0.98	0.98	0.96	0.87										
LN	0.39	0.93	0.98	0.99	0.94	0.97									
LFW	0.61	0.82	0.92	0.97	0.97	0.87	0.97								
PFW	0.44	0.92	0.98	0.99	0.97	0.95	0.99	0.98							
PR	0.54	0.82	0.92	0.94	0.99	0.84	0.90	0.93	0.94						
SC	0.40	0.27	0.33	0.43	0.30	0.40	0.50	0.53	0.44	0.17					
TR	0.53	−0.79	−0.65	−0.51	−0.40	−0.71	−0.52	−0.30	−0.50	−0.43	0.19				
CA	0.07	0.98	0.97	0.91	0.86	0.96	0.90	0.80	0.91	0.86	0.13	−0.81			
CB	0.05	0.98	0.96	0.91	0.85	0.96	0.89	0.79	0.90	0.85	0.13	−0.82	0.99		
CT	0.06	0.98	0.97	0.91	0.86	0.96	0.90	0.80	0.90	0.86	0.13	−0.81	1.00	0.99	
ANT	−0.77	0.58	0.37	0.24	0.04	0.50	0.28	0.02	0.22	0.04	−0.04	−0.89	0.53	0.55	0.54

Note: NTS—nitrogen total in soil, NLC—nitrogen content in leaves, CNU—canopy nitrogen uptake, RGR—relative growth rate, PH—plant height, SN—shoot numbers, LN—leaf numbers, LFW—fresh weight of leaves, PFW—plant fresh weight, PR—photosynthetic rate, SC—stomatal conductivity, TR—transpiration rate, CA—chlorophyll α, CB—chlorophyll β, CT—chlorophyll total.

2.2. Growth Performance under Different Pruning Levels and N Dosages

Growth performance seemed to be significantly affected by the single factor effect, rather than a combination of both N dosage and pruning factor (Table 2). The results of the analysis of correlation highlighted the positive, strong and significant correlation between relative growth rate (RGR) to plant height (r 0.97), number of shoots (r 0.96) and number of leaves (r 0.99) (Table 1). Another statistical analysis, namely DMRT, also reported that the RGR, plant height, and number of leaves were increased significantly, along with the increase in the dose of N fertilizer given. Plants treated with 40 g N per plant showed the best growth performance, especially compared to the control, by displaying significant improvements of about 99% on RGR, 48% on plant height, and 146% on leaf numbers. However, the best growth performance on the 40 g N plant^{-1} treatment was not significantly different to the 20 g N plant^{-1}.

Table 2. Kaffir lime growth performance under different pruning levels and nitrogen-fertilizer dosages.

Treatment	RGR (g week^{-1})	Plant Height (cm)	Shoot Number	Leaf Numbers
		Nitrogen fertilizer (N) factor		
0 g N	1.71 ± 0.27 c	53.75 ± 14.75 b	6.50 ± 1.05 b	52.50 ± 9.07 c
10 g N	2.39 ± 0.22 b	58.75 ± 13.65 b	7.50 ± 1.38a b	90.50 ± 8.55 b
20 g N	3.15 ± 0.43 a	79.13 ± 24.91 a	7.83 ± 1.72 a	117.33 ± 10.31 a
40 g N	3.40 ± 0.38 a	79.29 ± 23.27 a	8.67 ± 2.34 a	129.17 ± 8.40 a
		Pruning (P) factor		
SP (10 cm)	2.42 ± 0.65 b	51.87 ± 8.95 b	8.92 ± 1.56 a	92.92 ± 32.54 a
HP (30 cm)	2.90 ± 0.78 a	83.59 ± 19.41 a	6.33 ± 0.65 b	101.83 ± 30.69 a
N*P	Ns	Ns	Ns	Ns

Note: mean values within the same column and same factor followed by the same letter are not significantly different based on DMRT at α 0.05. RGR—relative growth rate, SP—short pruning, HP—high pruning, N*P—the interaction of nitrogen-fertilizer dosages and pruning levels, Ns—not significant.

Harvesting activity employing a high pruning type (leaving the main stem at 30 cm above the ground) stimulated greater support to subsequent kaffir lime growth, rather than the short pruning type (leaving the main stem at 10 cm above the ground). The high pruning type had 20% greater growth rate than the short ones. However, the short pruning type successfully induced more shoots than the high ones. In terms of leaf numbers, the result was not significantly different between the treatments.

2.3. Plant Production under Different Pruning Levels and N Dosages

The statistical analysis depicted an insignificant interaction effect of N dose and pruning on all the variables observed related to plant production. Kaffir lime production was solely influenced either by N dose or pruning. Concerning N-fertilizer dosage, the best results of plant fresh weight were observed in plants fertilized with 40 g N that experienced an increase of 55.13 g compared to control. However, it was not markedly different from those fertilized with 20 g N (Table 3). The fresh weight of plants fertilized with 10 g N was 30% higher than controls. However, it was still 25% lower than those fertilized with 40 g N. Concerning the pruning levels, the short-pruned type is thought to have a lower assimilated reserve than the high-pruned ones, leading to a lower production response. Pruning by leaving 30 cm of the main stem above the ground resulted in the improvement of plant and leaf production by about 28 and 22%, respectively, compared to the short pruning type.

The present experiment also revealed the partition of biomass in the entire plant body in response to N dose and pruning factor. In the absence of N fertilizer, the stem became the most dominant part, representing more than 50%. In the presence of N fertilizer, the more N applied, the more dominant the leaf biomass, exceeding the stem portion, in contrast to previous case (Figure 2A). Unlike the root and stem parts, the leaves are the most commercially valuable part of the kaffir plant. Therefore, the context of yield in kaffir lime is associated with the number and weight of leaves harvested in a certain unit of growing area. The estimation of leaf production extrapolated from the present findings, showed that plants fertilized with 40 g N and 20 g N received the best treatment, with an increase in yield of more than 2× compared to control (Figure 2B). In addition, regression analysis also re-confirmed and identified a strong association between (i) leaf fresh weight and N dose (Figure 3A) and (ii) leaf numbers and N dose (Figure 3B). The application of N fertilizers at various doses showed a quadratic pattern on both the fresh weight of leaves (R^2 = 0.9864) and leaf numbers (R^2 = 0.9998). The fresh weight of leaves in plants fertilized with 10 g N, 20 g N and 40 g N increased over controls, by 82, 174 and 198% respectively. The number of leaves in plants fertilized with 10 g N, 20 g N and 40 g N increased over controls, by 72, 123 and 146% respectively. However, there was no significant difference in fresh weight of leaves and number of leaves between 40 g N plant^{-1} and 20 g N plant^{-1}.

Table 3. Plant production (fresh weight) of kaffir lime under different pruning levels and nitrogen-fertilizer dosages.

Treatment	Fresh Weight (g)			
	Plant	Leaves	Stem	Root
		Nitrogen-fertilizer (N) factor		
0 g N	76.36 ± 13.18 c	19.33 ± 3.65 c	39.29 ± 8. 21 c	17. 75 ± 1.73 c
10 g N	99.10 ± 11.74 b	35.22 ± 3.67 b	44.69 ± 7. 53 b	19. 18 ± 1. 72 bc
20 g N	124.51 ± 20.15 a	52. 99 ± 9. 46 a	51. 94 ± 9. 82 a	21.31 ± 1. 83 ab
40 g N	131.53 ± 16.56 a	57.23 ± 5. 59 a	50. 21 ± 10.91 a	22. 37 ± 1.43 a
		Pruning (P) factor		
SP (10 cm)	94.71 ± 20.98 b	37. 12 ± 14. 67 b	38. 46 ± 4. 44 b	19. 13 ± 2. 36 b
HP (30 cm)	121.03 ± 25.94 a	45. 26 ± 17. 66 a	54.61 ± 6.80 a	21. 16 ± 2. 10 a
N*P	Ns	Ns	Ns	Ns

Note: mean values within the same column and same factor followed by the same letter are not significantly different based on DMRT at α 0.05. SP—short pruning, HP—high pruning, N*P—the interaction of nitrogen-fertilizer dosages and pruning levels, Ns—not significant.

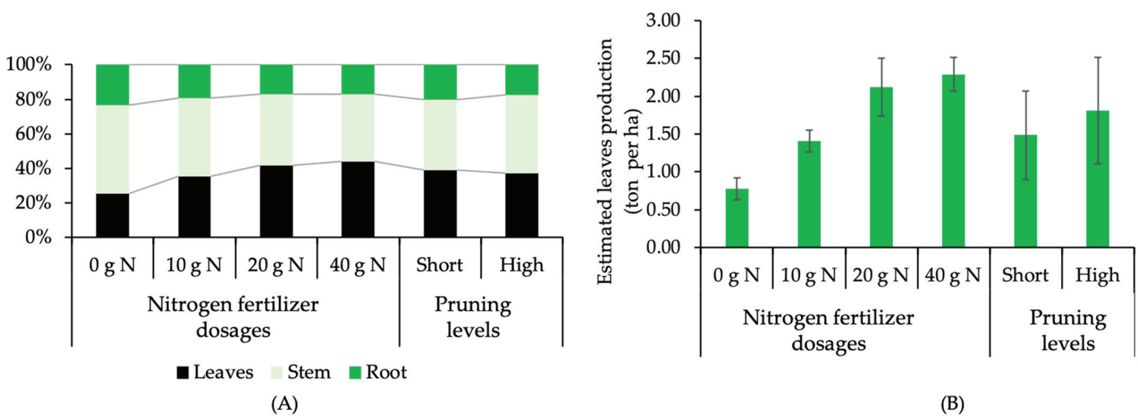

Figure 2. (**A**) Biomass partition and (**B**) estimated leaf production (ton per ha) of kaffir lime under different nitrogen-fertilizer dosages and pruning levels.

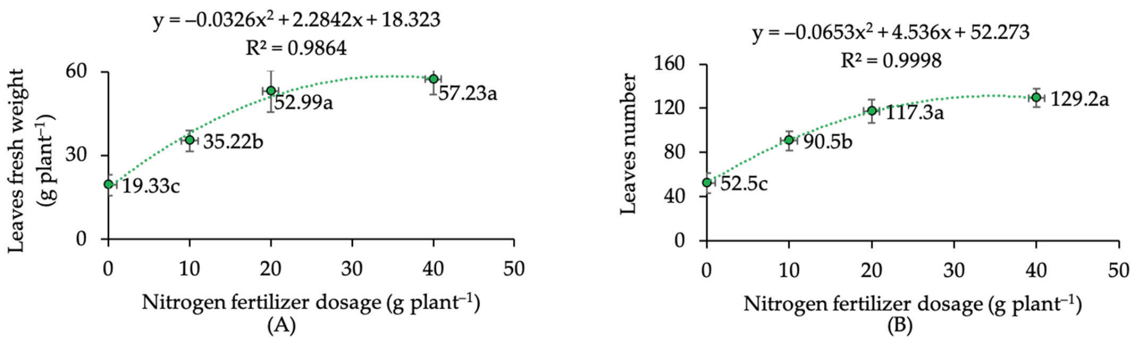

Figure 3. Regression curve of kaffir lime (**A**) fresh weight of leaves and (**B**) leaf numbers under different nitrogen-fertilizer dosages. Note: mean values followed by the same letter within the same curve are not significantly different based on DMRT at α 0.05.

2.4. Physiological Response of Kaffir Lime under Different Pruning Levels and N Dosages

The alteration of growth and production of kaffir lime in response to different N-fertilizer dosages and pruning levels was followed by the variable of plant physiology. Higher leaf production in N-fertilized plants was associated with an increase in the rate of plant photosynthesis. The rate of photosynthesis between plants fertilized with 20 g N and 40 g of N was insignificant; however, there was a noticeable increase of about 15% compared to both control (0 g N) and 10 g N (Table 4). In contrast, there was no significant difference in N-fertilizer dosage on stomatal conductance, transpiration rate, stomatal limitation to CO_2 uptake, and water-use efficiency (WUE) in the kaffir lime plants. Stomatal conductance, transpiration rates, intrinsic WUE and instantaneous LUE in the present experiment varied in the range of 0.37–0.38 mol H_2O m^{-2} s^{-1}, 6.25–6.59 mmol H_2O m^{-2} s^{-1}, 2.68–3.23 µmol CO_2 mmol H_2O^{-1}, and 0.58–0.98 µg lux^{-1}, respectively.

Table 4. Kaffir lime physiological response under different pruning levels and nitrogen-fertilizer dosages.

Treatment	Pn	Tr	Sc	WUE	LUE
			Nitrogen-fertilizer (N) factor		
0 g N	17.52 ± 1.19 b	6.54 ± 1.56	0.371 ± 0.09	2.68 ± 0.76	0.58 ± 1.56
10 g N	17.63 ± 1.22 b	6.50 ± 1.85	0.383 ± 0.12	2.74 ± 0.66	0.74 ± 1.85
20 g N	20.03 ± 1.13 a	6.59 ± 1.39	0.381 ± 0.09	3.04 ± 0.81	0.92 ± 1.39
40 g N	20.20 ± 0.99 a	6.25 ± 0.96	0.376 ± 0.06	3.23 ± 1.03	0.98 ± 0.96
			Pruning (P) factor		
H10	18.69 ± 1.81	6.70 ± 1.64	0.397 ± 0.10 a	2.81 ± 1. 10	0.71 ± 1.64
H30	18.99 ± 1.59	6.24 ± 1.22	0.358 ± 0.08 b	3.04 ± 1. 30	0.90 ± 1.22
N*P	Ns	Ns	Ns	Ns	Ns

Note: mean values within the same column and same factor followed by the same letter are not significantly different based on DMRT at α 0.05. Pn—photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), Tr—transpiration rate (mmol H_2O m^{-2} s^{-1}), Sc—stomatal conductance (mol H_2O m^{-2} s^{-1}), Sl—stomatal limitation to CO_2 uptake (mol m^{-2} s^{-1}), WUE—intrinsic water-use efficiency (µmol CO_2 mmol H_2O^{-1}), LUE—instantaneous light-use efficiency (µg lux^{-1}). Ns—not significant, N*P—the interaction of nitrogen-fertilizer dosages and pruning levels.

As the only variable that was significantly affected by N-fertilizer dose, the rate of photosynthesis in kaffir lime was strong and positively correlated to the content of chlorophyll, i.e., chlorophyll a (r 0.86) and chlorophyll b (r 0.85) (Table 1). The chlorophyll content increased along with the increase in N fertilizer applied to kaffir lime plants and was actually supported by the morphological fact that could be seen directly in the field. Based on field observations, the yellowish color on kaffir lime leaves fertilized with 0 g N and 10 g $plant^{-1}$ was an early symptom of N-deficiency stress. Meanwhile, plants with 20 g N and 40 g N plant^{-1} experienced normal green leaves, presumably not experiencing a N-deficiency condition (Figure 4). Such external leaf color variations between the N-fertilizer dosages used could be reconfirmed by the results of pigment analysis.

Figure 4. Kaffir lime appearance under different pruning levels and nitrogen-fertilizer dosages.

The statistical analysis of the pigment content of kaffir lime revealed the significant effect of N dosage on plant chlorophyll content, both chlorophyll α and chlorophyll β. The absence of N-fertilizer application on the control treatment showed the lowest pigment chlorophyll content of all (Table 5). The higher the N-fertilizer dose applied, the higher the content of chlorophyll, chlorophyll β and chlorophyll total in kaffir lime leaves. In the 40 g N treatment, the content of chlorophyll α and chlorophyll β and its total chlorophyll increased significantly, by about 1.37 mg g^{-1}, 0.48 mg g^{-1}, respectively and 1.85 mg g^{-1} compared to the lime without fertilizer.

Table 5. Kaffir lime leaves' pigment content under different nitrogen-fertilizer dosages.

Treatment	Chlorophyll α (mg g^{-1})	Chlorophyll β (mg g^{-1})	Chlorophyll Total (mg g^{-1})	Anthocyanin (mg 100 g^{-1})
0 g N	0.319 ± 0.12 d	0.122 ± 0.06 d	0.441 ± 0.17 d	0.063 ± 0.04 a
10 g N	0.618 ± 0.08 c	0.229 ± 0.02 c	0.846 ± 0.10 c	0.077 ± 0.01 a
20 g N	0.953 ± 0.06 b	0.337 ± 0.02 b	1292 ± 0.09 b	0.051 ± 0.01 a
40 g N	1.687 ± 0.12 a	0.603 ± 0.04 a	2.291 ± 0.16 a	0.088 ± 0.01 a

Note: mean values within the same column followed by the same letter are not significantly different based on DMRT at α 0.05.

3. Discussion

Pruning is the agricultural technique used to regulate plant growth, reduce pest and disease incidence, and increase horticultural management effectiveness [27–31]. Canopy rejuvenation is the foremost important benefit obtained from such a technique. In a rejuvenated canopy, new leaves grew to immediately restore the lost foliage [32] and these leaves possess much more productivity [33] due to the higher potential of carbon assimilation [32]. A previous study reported the use of light pruning in the form of pinching to induce robust canopy growth in early seedlings of kaffir lime [17]. In the postpruning period, the massive growth of lateral shoots is caused by lowering apical dominance [34–36]. In the fruit-oriented major citrus of mandarin, heavy pruning was applied to rejuvenate the canopy, but as a result, there was a decline of vegetative growth due to a severe decline in plant resource capacity [18]. Thus, mild to moderate pruning was highly recommended for those kinds of citrus [37,38]. However, to harvest the leaf yield of kaffir lime, growers have to apply heavy pruning. Due to the nature of citrus plants which are sensitive to leaf disturbance [17,18], the pruning level should be adjusted to be lighter. Comparative analysis on the pruning factor resulted in a significant increase in growth rate, plant and leaf production by about 20, 28 and 22%, respectively, in the high pruning compared to the short pruning that left only 10 cm of the main stem. The higher assimilate reserve in the existing main stem could likely support a higher growth rate, and final yield, regardless of nitrogen doses. Therefore, the recommendation for pruning in kaffir lime is the high pruning type by leaving the main stem 30 cm above the grafted join spot.

Aside from pruning, nitrogen management was also evaluated to provide the first N-fertilizer recommendations for kaffir lime grown under artificial mild shading. Most citrus growers use leaf tissue rather than soil as the basis for fertilizer application. Leaf tissue is a more representative proxy for estimating a tree's nutritional status for mobile nutrients, such as nitrogen [39–41]. The result of the leaf-based nutritional test should be compared with the optimal range of that nutrient [42,43]; thus, there is an urgency to estimate the optimal range of nutrients for achieving a profitable citrus yield [44]. A previous study [45] found the variation in optimal leaf nutrient contents in four fruit-oriented popular citrus, namely oranges, mandarin, grapefruit and pomelo. The existence of variations in the optimal leaf nutrient content is thought to be related to differences in cultural practices, edaphic, climatic, and genetic factors [44]. In fact, leaf nutrient-based citrus fertilizer recommendation guidelines have been intensively studied for seventy years in the USA [46–48], and have been updated several times by numerous researchers [49–51].

Concerning leaf nitrogen, published studies have produced recommendations based on total leaf-N concentration for popular fruit-oriented citrus species [45,50,52]. Interestingly, the present study proposed the total leaf N range (2.06–2.36% N total) for the leaf-oriented minor citrus, kaffir lime, grown under mild shading. The correlation and regression analysis were adopted in the present experiment since earlier studies frequently used it to estimate the relationship between citrus yield and leaf nutrient concentrations [45,50,53]. Our findings highlighted the positive and strong correlation between leaf-N status and relative growth rate (r 0.93) and final leaf numbers harvested (r 0.93). Regression analysis also found positive quadratic patterns between (i) N-fertilizer dose and fresh weight of leaves (R^2 0.9864) and (ii) N-fertilizer dose and leaf numbers (R^2 0.9998).

Due to its quadratic pattern, a dose of 40 g N plant^{-1} does not automatically become the best fertilizer recommendation. It has already been reported that plants fertilized with both 40 g N and 20 g N doubled the yield of the control. However, 20 g N plant^{-1} is more efficient with a relatively similar effect to 40 g N plant^{-1} for increasing growth rate, photosynthetic rate, fresh weight of leaves and leaf numbers of kaffir lime under mild-shading conditions. Mild shading was previously reported to produce a beneficial stress instead of a harmful one [17]. Best practice of N-fertilizer application under lightly shaded conditions may become a combo booster for kaffir lime leaf production. Similarly, the success of N fertilizer and beneficial shading for boosting plant growth performance was also reported by previous researchers, as indicated by larger and broader leaves [54], more dominant vegetative growth [55], and higher yield [56]. In contrast, slower plant growth leading to lower production performance was observed both in the control and the 10 g N plant^{-1} treatment. Vegetative growth inhibition is a common plant response under N-deficient conditions [57].

Aside from vegetative improvement, another advantage of best N-fertilizing practice is the regulation of assimilate translocation priority. N adequacy seemed to alter the assimilate translocation priority in kaffir lime plants. A N-sufficient plant, treated with both 20 g N plant^{-1} and 40 g N plant^{-1}, showed a dominant portion of leaves, while a deficient plant likely had a large portion of stem. Concerning agribusiness profit, the leaf part of the kaffir lime is more valuable and profitable than the stem or even the root [7,9,10], due to the content of various beneficial phytochemicals such as citronellal, citronellol, citronellyl acetate, linalool and caryophyllene that contribute to the strong aromatic formed [12,58,59].

The N-sufficient condition in the 20 g N plant^{-1} and 40 g N plant^{-1} displayed a good leaf–N total (>2%) associated with proper growth performance. That growth improvement is mainly caused by a higher photosynthetic rate and chlorophyll content. Similarly, the relationship between RGR to photosynthetic and chlorophyll content was confirmed by the correlation results, with coefficients of correlation of about 0.94 and 0.91, respectively. A N-sufficient status is vital for constructing optimal leaf photosynthesis. The leaf-N status represents the protein content for the Calvin cycle and thylakoids that are subsequently associated with leaf photosynthetic capacity [60]. Concerning the normal leaf cells of C_3 plants, including kaffir lime, N is allocated mostly in the chloroplast, at about 75%, with 10% in the cell wall, and 5% in mitochondria [61]. The variation of those partitions may occur in N-deficient conditions.

The N-deficient plant, as observed in the control and 10 g N plant^{-1}, displayed a low leaf–N total (<2%) and exhibited a yellow leaf appearance, implying a chlorosis phenomenon. The data of the chlorophyll test also displayed a significant reduction compared to the N-sufficient plant. Leaf chlorophyll content was previously reported to be crucial for assimilation rate since it positively and strong correlates to leaf photosynthetic rate [62]. The degradation of chlorophyll, called chlorosis, begins to appear on the lower leaves prior to spreading over the entire canopy, and even in severe cases, it can cause necrosis on old leaves [57]. Chlorosis, as a popular N-deficient symptom, is caused by a failure to form chlorophyll pigments [63]. A published study in oranges and pomelo described the main reason behind lowering CO_2 assimilation, i.e., impairment of the thylakoid structure and photosynthetic electron transport chain (PETC) in the leaves and declining leaf photosynthetic pigment levels [64]. Moreover, N-deficient leaves are proven to have smaller chloroplasts and no starch granules. In contrast, N-sufficient leaves have large chloroplasts, with fully formed grana components and larger starch granules for performing greater assimilation [65]. In contrast, mild-shaded and N-fertilized plants may have a more robust photosynthetic apparatus, as evidenced by a large number of thylakoids per granum and an abundance of grana per chloroplast [54].

4. Materials and Methods

4.1. Study Site

The experiment was carried out at Pasir Kuda experimental field of IPB University, Bogor, Indonesia (6°36′36″ S, 106°46′47″ E, 263 m above sea level) from November 2018 to March 2019. The soil description of the Pasir Kuda experimental field was a sandy clay latosol soil with an actual pH, C-organic, N total, P total, and K total of about 6.7, 2.37%, 0.19%, 240 mg P_2O_5 100 g^{-1}, and 160 mg K_2O 100 g^{-1}. During the study period, the experimental field was exposed to the rainy season, with monthly rainfall intensity ranging from 230 mm to 318 mm (x-bar 289 mm).

4.2. Planting Materials

Plant materials were nine-month-old seedlings obtained by the grafting technique that combined kaffir lime (*Citrus hystrix* DC) scions onto rangpur lime (*C. limonia* Osbeck) rootstock. Before field transplanting, seedlings underwent initial selection to confirm only selected plants were involved in the present experiment, with certain requirements, such as bifoliate leaves, pest and disease-free, normal growth, dormant apical bud, uniform in leaf numbers (30 ± 2 leaves) and plant height (60 ± 4 cm).

4.3. Research Procedure

The present study employed a split-plot experimental design, with N dosage as the main plot and pruning level as the subplot. Four levels of N dosage were tested, viz., 0 g N plant^{-1}, 10 g N plant^{-1}, 20 g N plant^{-1}, and 40 g N plant^{-1}. Two levels of pruning were also evaluated, namely short and high pruning. Short pruning was technically defined as leaving only 10 cm of the main stem above the ground, whereas high pruning gave the cutting point at 30 cm from above the ground. Six replications were provided for each combination treatment; thus, 46 experimental units in the form of kaffir lime seedlings were counted in total.

Kaffir lime seedlings were raised in a monoculture cropping system under mild-shading conditions that were artificially formed by installing a black shading net 2 m above the soil surface. In a previous, similar study, this treatment resulted in (1) a reduction of sunlight, ambient temperature, and soil temperature of about 23, 6.3, and 6.5%, respectively; and (2) the improvement of ambient relative humidity by about 2%, compared to open field monoculture system [17]. Kaffir lime transplanting to the field was conducted in November 2018, with a 50 cm × 50 cm planting distance.

Pruning was applied, according to the treatment, in December 2018, or 30 days after planting (DAP). The cutting point for pruning was actually determined based on the grafted join spot. That spot was normally found 15 cm above the stem base of the rootstock variety. However, the joining spot seemed to equal the soil surface due to the soil banking technique for suppressing undesired shoot growth from the rootstock variety.

Inorganic fertilizers, apart from N, such as phosphorus (P) and potassium (K) were applied uniformly at 33 DAP, in the form of 15 g P_2O_5 and 10 g K_2O, following the national citrus agency recommendations [26]. N fertilization was carried out simultaneously with P and K, with a dose adjusted to the treatment. All mentioned fertilizer was delivered in the morning (7.00 am) of a sunny day through a soil drench surrounding the seedling (10 cm away from the main stem). Hand-weeding was routinely applied every month. Pest and disease inspection was conducted weekly, and the damage was chemically managed. Harvesting was scheduled at 120 DAP in March 2019.

4.4. Measured Variables

Measured variables were N status in soil and leaves, canopy N uptake, growth performance, plant production (fresh weight), and physiological responses. The N content in the soil and leaf samples was analyzed by using Kjeldahl method at 115 DAP. Plant production (fresh weight) was measured on harvesting day (120 DAP) by weighing either the whole plant or individual parts in the analytical balance (Hwh, China). Plant samples were then dried using an oven at 80 °C for 3 days to obtain plant dry weight by using a similar analytical balance. Canopy N uptake was obtained from the multiplication of the canopy dry weight and the N content of leaves. The relative growth rate (RGR) was calculated based on the ratio of the increase in plant dry weight to the number of weeks from the 1st pruning (at 30 DAP) to the 2nd pruning (120 DAP), i.e., 13 weeks. Plant height, shoot number, and leaf numbers were also observed on harvest day using a roll meter (Kenmaster, North Jakarta, Indonesia), and hand counter (Kenko, North Jakarta, Indonesia), respectively.

Plant physiological variables such as photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) and transpiration rate (mmol H_2O m^{-2} s^{-1}) were measured at 94 HSP at 10.00 am (sunny day) using the Li-6400XT portable photosynthesis system (Licor Inc, Lincoln, NE, USA). In addition, the present experiment also measured (i) intrinsic water-use efficiency (μmol CO_2 mmol H_2O^{-1}) and (ii) instantaneous LUE (μg lux^{-1}) by (i) dividing the rate of photosynthesis by the rate of transpiration rate and (ii) dividing the fresh weight of leaves by the perceived sunlight amount, respectively. The amount of perceived sunlight was measured by Lux-28 portable digital lux meter (Danoplus, Hong Kong, China). Leaf pigment was measured at 86 DAT in the present experiment by following the Sims and Gamon method [66].

4.5. Data Analysis

Quantitative data obtained in the present experiment was subjected to the analysis of variance (ANOVA) and any significant difference found was further analyzed by the Duncan's multiple range test (DMRT) at α 0.05. Pearson correlation analysis was carried out to find the closeness of the relationship between the observed variables, such as N status, plant growth, production, and physiological response. In addition, regression analysis was also performed to elucidate the association between N dose and certain important yield-related variables, such as canopy N uptake, fresh weight of leaves and leaf numbers. All statistical analysis was performed using the Statistical Tool for Agricultural Research (STAR) version 2.0.1.

5. Conclusions

The present experiment succeeded in determining the best practice for pruning and N fertilizer to boost leaf production under mild-shading conditions. Pruning by leaving 30 cm of the main stem above the ground resulted in a significant improvement of plant and leaf production by about 28 and 22%, respectively, compared to the short pruning that left only 10 cm of the main stem. The higher assimilate reserve in the existing main stem likely produced higher support for recovering the lost foliage. Concerning N management, N-fertilizer dosage had a noticeable effect on the total N content of the leaf tissue, with the highest N–leaf tissue content achieved from the highest N dose. Both correlation and regression analysis confirmed that N is crucial for plant growth and plant yield due to the role of this nutrient in chlorophyll content and photosynthetic rate. Kaffir lime treated with 0 and 10 g N plant^{-1} experienced N-deficient conditions, as indicated by leaf chlorosis, leading to lower chlorophyll content, photosynthetic rate, relative growth rate and then leaf yield harvested. A N-sufficient condition was achieved as the effect of 20 and 40 g N plant^{-1} application, producing a great growth and yield performance due to a high carbon assimilation rate. However, a dose of 40 g N plant^{-1} does not automatically become the best fertilizer recommendation, since 20 g N plant^{-1} is more efficient with a relatively similar effect for increasing kaffir lime leaf production.

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Productivity and Nutrient Balance of an Intensive Rice–Rice Cropping System Are Influenced by Different Nutrient Management in the Red and Lateritic Belt of West Bengal, India

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Abstract: Rice is the lifeline for more than half of the world population, and in India, in view of its huge demand in the country, farmers adopt a rice–rice cropping system where the irrigation facility is available. As rice is a nutrient-exhausting crop, sustainable productivity of rice–rice cropping system greatly depends on appropriate nutrient management in accordance with the inherent soil fertility. The application of an ample dose of fertilizer is the key factor for maintaining sustainable rice yields and nutrient balance of the soil. Considering the above facts, an experiment was conducted on nutrient management in a rice–rice cropping system at the university farm of Visva-Bharati, situated in a sub-tropical climate under the red and lateritic belt of the western part of West Bengal, India, during two consecutive years (2014–2016). The experiment was laid out in a Randomized Completely Block Design with 12 treatments and three replications, with different rates of N:P:K:Zn:S application in both of the growing seasons, namely, *kharif* and *Boro*. The recommended (ample) dose of nutrients was 80:40:40:25:20 and 120:60:60:25:20 kg ha^{-1} of N:P_2O_5:K_2O:Zn:S in the *Kharif* and *Boro* season, respectively. A high yielding variety, named MTU 7029, and a hybrid, Arize 6444 GOLD, were taken in the *Kharif* and *Boro* seasons, respectively. The results clearly indicated that the application of a recommended dose of nutrients showed its superiority over the control (no fertilizer application) in the expression of growth characters, yield attributes, yields, and nutrient uptake of *Kharif* as well as *Boro* rice. Out of the all treatments, the best result was found in the treatment where the ample dose of nutrients was applied, resulting in maximum grain yield in both the *Kharif* (5.6 t ha^{-1}) and *Boro* (6.6 t ha^{-1}) season. The corresponding yield attributes for the same treatment in the *Kharif* (panicles m^{-2}: 247.9; grains panicle^{-1}: 132.0; spikelets panicle^{-1}: 149.6; test weight: 23.8 g; and panicle length: 30.6 cm) and *Boro* (panicles m^{-2}: 281.6; grains panicle^{-1}: 142.7; spikelets panicle^{-1}: 157.2; test weight: 24.8 g; and panicle length: 32.8 cm) season explained the maximum yield in this treatment. Further, a reduction or omission of individual nutrients adversely impacted on the above traits and resulted in a negative balance of the respective nutrients. The study concluded that the application of a recommended dose of nutrients was essential for proper nutrient balance and sustainable yields in the rice–rice cropping system.

Keywords: nutrient management; rice–rice cropping system; growth characters; yield attributes; productivity; nutrient uptake and balance

1. Introduction

Rice is one of the most important cereals consumed across the globe and grown in different environmental conditions. A rice–rice cropping system is usually practiced by farmers where sufficient irrigation is available or in favorable lowland rainfed areas [1,2]. Apart from irrigation availability, high consumer demand, a relatively stable market price, and assurance of a minimum support price by the government encourage the farmers to grow two rice crops continuously in consecutive seasons. Though the rice–rice system seems to be feasible from a farmer's perspective, cereal–cereal cropping systems are often considered unsustainable and are discouraged [3] in terms of nutrient balance in the soil as well as agricultural sustainability [4]. Rice, being a nutrient intensive crop, absorbs a high amount of nutrients. Thus, a rice–rice system is expected to be even more nutrient exhaustive. Unless proper nutrient management practices are followed, soils may develop severe nutrient deficiency over a period of time, negatively affecting agricultural sustainability [5]. Rice–rice cropping systems are most prevalent across a major portion of India as well as South Asia, especially among small and marginal farmers.

Nitrogen (N), phosphorus (P), and potassium (K) are considered as primary nutrients and are very important for the growth and development of rice [6]. Nitrogen is responsible for vegetative growth, improving the leaf area index, chlorophyll synthesis, and so on [7]; thus, increasing photosynthesis and assimilate production in plants. N is deficient in most of the rice-growing areas, which requires a proper focus on nitrogen nutrition [8]. Phosphorus is known for its role in root growth, root development, and reproduction [9]. P is also known to improve tillering and promotes early flowering. Potassium, though not a constituent of organic structures of plants, is very important for plant strength, resistance to biotic and abiotic stresses, and stomatal activity [10]. In addition to primary nutrients, sulphur (S), a secondary plant nutrient, also plays a vital role in plant growth and development as S performs its distinctive role in protein and chlorophyll synthesis [11]. In addition to macronutrients, rice crop also requires micronutrients for completing its life cycle and proper nutrition. Among the different micronutrients, deficiency in zinc (Zn) is commonly observed in rice-growing areas [12], where close to 50% of soils in rice-growing tracts are deficient in Zn [13]. Zinc takes part in the carbohydrate transformation and it is an essential constituent of enzymes such as carbonic anhydrase, superoxide dismutase, and alcohol dehydrogenase [14]. Zinc is also involved in the auxin biosynthesis process. Soil submergence, which is commonly practiced in rice cultivation, results in a deficiency of Zn. Unlike macronutrients, the availability of Zn is higher at a low pH. Alkaline or calcareous soils may result in Zn deficiency [15].

A rice–rice cropping system, when practiced, removes nutrients from the same soil depth continuously. If the crops cultivated in a cropping system have a similar nutrient demand and the removal pattern of nutrients from the soil is also the same, then, unless proper care is taken to replenish the nutrient taken up by the crop, a single or multiple nutrient deficiencies may develop over a period of time [16]. Understanding the role of different nutrients in the growth and yield of rice is essential to provide essential nutrients in the required quantity to obtain higher productivity. However, higher productivity should also be sustainable to achieve long-term food security goals.

Imbalanced nutrient application is one of the most important reasons for multi-nutrient deficiency [17]. As the application of nitrogen increases plant dry matter production, a high amount of nitrogen is also expected to increase the uptake of other nutrients.

Unless a sufficient amount of other nutrients is applied under such conditions, the crop will continuously drain the native soil nutrients. This, when practiced continuously over years, causes a deficiency in nutrients. Application of nutrients in adequate amounts and suitable proportion is the key to crop nutrition. As the application of all the essential nutrients is practically impossible, those nutrients whose deficiency is prevalent or the nutrients which are yield limiting should be given priority in the nutrient management plan. In addition to those nutrients, nutrients that are expected to be deficient due to huge removal by crops over years in a particular cropping system must be replenished regularly to avoid the development of new nutrient deficiencies. For understanding these phenomena, knowledge regarding the role of important nutrients such as N, P, K, S and Zn in crop growth and yield should be considered. The nutrient balance in the cropping system also should be studied to understand the nutrient removal pattern of the crops under different nutrient combinations.

The soil and agro-climatic conditions of the red and lateritic belt are unique, and the rice-based cropping system is predominant in the region. The improvement of irrigation facilities and adaptation of HYVs and hybrids attracted farmers to adopt a rice–rice cropping system. As this cropping system is nutrient exhaustive, the development of multi-nutrient deficiency has been observed in recent times [18], drawing the attention of researchers. Similar observations on fertility degradation due to the rice-based cropping system were also noted in Southeast Asia [19]. Under these circumstances, there is an urgent need for balanced nutrient management in intensive cropping systems as a cost-effective and environmentally friendly approach to achieve agricultural sustainability in the region. Taking into consideration the above facts, an experiment was performed to evaluate the impact of different nutrients (inclusive of omission of specific nutrients) management on the growth and productivity of rice in a rice–rice cropping system. The uptake and nutrient balance are also studied to understand the necessity of nutrient supplementation to avoid long-term nutrient deficiencies in a rice–rice cropping system.

2. Materials and Methods

2.1. Experimental Site

The site of the field trial was the university farm of Visva Bharati (20°39′ N latitude and 87°42′ E longitude, with an altitude of 58.9 m above M.S.L.), situated in a sub-tropical climate under the red and lateritic belt of the western part of West Bengal, India [20]. The soils of the field trial were sandy loam soil belonging to the typical Ultisols. The characteristics and initial fertility of the experimental soil are described in Table 1.

Table 1. Characteristics and initial fertility of the experimental soil and methodologies followed for determination of soil quality.

Particulars	Characters/Value	Status	Methodology	References
Texture	Sandy loam	-	Hydrometer method	[21]
pH	5.65	Acidic	Determined by pH meter in 1:2.5 ratio of soil–water suspension	[22]
Electrical conductivity (EC) (dS m^{-1})	0.26	-	Solubridge method	[22]
Organic carbon (%)	0.35	Low	Walkley and Black method	[22]
Available nitrogen (kg ha^{-1})	230.0	Low	Alkaline permanganate method	[23]
Available phosphorous (kg ha^{-1})	11.2	Low	Bray's method	[24]
Available potassium (kg ha^{-1})	125.2	Medium	Flame photometer method	[25]
Zinc (mg kg^{-1})	0.22	Low	Diethylenetriaminepentaacetate (DTPA) extractable Zn determination by Atomic Absorption spectroscopy (AAS)	[26]
Sulphur (kg ha^{-1})	10.5	Low	Turbidimetric Method	[27]

The location falls in the region of the southwest monsoon, and monsoon rains generally start from the end of June and continue up to mid-October, with an average annual rain of 1377 mm. Out of the total annual rain, monsoon rain constitutes about 80–90%. The meteorological information, such as the maximum and minimum temperature (°C), rainfall (mm), and relative humidity (%) during the period of experimentation (July 2014–June 2016), were received from the meteorological observatory of the Institute of Agriculture, Sriniketan, and is presented in Figure 1.

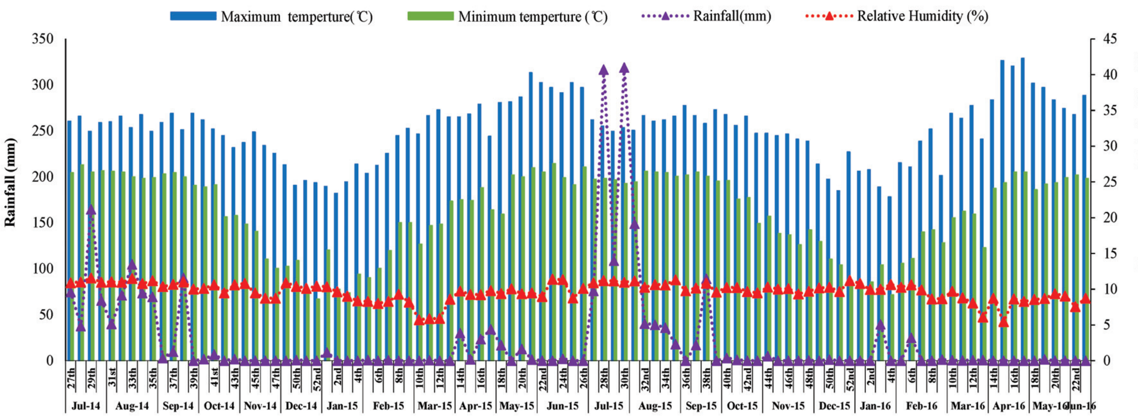

Figure 1. Meteorological data during the crop season (July 2014 to June 2016).

2.2. Experimental Design and Treatments

The experiment on nutrient management in the rice–rice cropping system was carried out for two years (four cropping seasons): 2014–2015 and 2015–2016. The experiment was laid out in a Randomized Complete Block Design (RCBD) with twelve treatments (net plot area of 5 m × 4 m each) and we repeated all treatments three times. The treatments were in the *Kharif* season: T_1: $N_{80}P_{40}K_{40}Zn_{25}S_{20}$; T_2: $N_{40}P_{40}K_{40}Zn_{25}S_{20}$; T_3: $N_0P_{40}K_{40}Zn_{25}S_{20}$; T_4: $N_{80}P_{20}K_{40}Zn_{25}S_{20}$; T_5: $N_{80}P_0K4_0Zn_{25}S_{20}$; T_6: $N_{80}P_{40}K_{20}Zn_{25}S_{20}$; T_7: $N_{80}P_{40}K_0Zn_{25}S_{20}$; T_8: $N_{80}P_{40}K_{40}Zn_{12.5}S_{20}$; T_9: $N_{80}P_{40}K_{40}Zn_0S_{20}$; T_{10}: $N_{80}P_{40}K_{40}Zn_{25}S_{10}$; T_{11}: $N_{80}P_{40}K_{40}Zn_{25}S_0$; and T_{12}: control, (without any fertilizer); whereas in the *Boro* season: T_1: $N_{120}P_{60}K_{60}Zn_{25}S_{20}$; T_2: $N_{60}K_{60}Zn_{25}S_{20}$; T_3: $N_0K_{60}Zn_{25}S_{20}$; T_4: $N_{120}P_{30}K_{60}Zn_{25}S_{20}$; T_5: $N_{120}P_0K_{60}Zn_{25}S_{20}$; T_6: $N_{120}P_{60}K_{30}Zn_{25}S_{20}$; T_7: $N_{120}P_{60}K_0Zn_{25}S_{20}$; T_8: $N_{120}P_{60}K_{60}Zn_{12.5}S_{20}$; T_9: $N_{120}P_{60}K_{60}Zn_0S_{20}$; T_{10}: $N_{120}P_{60}K_{60}Zn_{25}S_{10}$; T_{11}: $N_{120}P_{60}K_{60}Zn_{25}S_0$; and T_{12}: control (without any fertilizer). The recommended (ample) dose of nutrients was 80:40:40:25:20 and 120:60:60:25:20 kg ha^{-1} of $N:P_2O_5:K_2O:Zn:S$ in the *Kharif* and *Boro* season rice, respectively, and the treatment T_1 received an ample dose of nutrients in both the seasons. In the case of T_2, P, K, S, and Zn were applied in an ample dose, and 50% of the N was applied. In T_3, N, K, S, and Zn were applied with an ample dose, 0% of N applied, and the same manner was applied for the remaining treatments up to T_{11}, but in T_{12}, no fertilizer was applied and considered as the control. The total amount of P, K, Zn, and S were applied as basal, while nitrogen was applied in three splits. The HYV and hybrid of rice were taken in the *Kharif* and *Boro* season with the same duration (Table 2).

Table 2. Variety/hybrid chosen, date of transplanting, and duration of rice (2014–2016).

Particulars	*Kharif*	*Boro*
Cropping system	Rice	Rice
Variety/hybrid	HYV (High yielding variety) rice variety: MTU 7029	Hybrid rice: Arize 6444 GOLD
Date of transplanting	3 August 2014; 2 August 2015	2 February 2015 and 2016
Duration	150 days	150 days

2.3. Cultural Practices

The standard procedure of rice cultivation in the locality was adopted for both seasons. The treated seeds (with Carbendazim at the rate of 2 g kg^{-1} of seed) were sown in the nursery during both seasons and the seeds were covered lightly with soil. For the main field preparation, the soil was first tilled thoroughly cross wise with a tractor-drawn harrow at an optimum moisture condition. Then tillage was done with a mouldboard plough (25 cm deep) to obtain a good tilth and it was followed by planking. The clods and stubbles of previous crops were removed from the land. The field was flooded with water and the puddling was done under saturated moisture conditions prior to three days of transplanting. After proper levelling, the field was laid out by making net plots (5 m $\times$ 4 m each), plot bunds, and channels for irrigation and drainage. After completion of the layout, nutrients were applied as per the treatments. The sources of nitrogen, phosphate, potash, sulphur, and zinc were urea, diammonium phosphate (DAP), muriate of potash (MOP), Bentonite-S (90% of S), and Zn-Ethylenediamine tetraacetic acid (EDTA), respectively. Among the different fertilizers, nitrogen was applied in splits. Half of the nitrogen and a full quantity of the other nutrients were applied as the basal treatment; however, the rest of the N was top-dressed in two equal splits during the maximum tillering and panicle initiation stages for *Kharif* as well as for *Boro* rice.

The 21-day old seedlings were transplanted in the main field at a spacing of 20 cm $\times$ 15 cm. In each hill, three seedlings were transplanted. The weeds were removed by hand weeding at early tillering (20 days after transplanting (DAT) and late tillering (40 DAT)). After transplanting, the field was kept saturated with moisture for three weeks to facilitate tillering and followed by a water stagnation of 5 $\pm$ 2 cm was maintained up to physiological maturity. Before topdressing of N, standing water was removed from the rice field and irrigated again on the next day and water stagnation was maintained. Ten days prior to harvest, stagnant water was removed. In the *Kharif* season, four irrigations were applied, whereas the in *Boro* season, the crop required six irrigations. The crop faced a mild attack of yellow stem borer and recommended protocols of the university were adopted to manage the pest. However, crop damage due to pest attacks were negligible. The crop was harvested from each net plot manually when it reached 80% maturity. The harvested crop was threshed, winnowed, and the sun-dried weight was recorded at 12% moisture.

2.4. Measurements and Analytical Procedures

2.4.1. Growth and Yield Attributes

The third rows from the border of each side of a plot were sampled to record biometric observations. Different growth characters, namely, plant height, dry matter accumulation, leaf area index (LAI), and number of tillers were recorded at different growth stages (20, 40, 60, 80, 100, and 120 days after transplanting, DAT) and the crop growth rate (CGR) was calculated for different periods of the rice–rice cropping system for two consecutive years. For measurement of dry matter accumulation, five randomly selected plants were taken as a destructive sample; the leaves were separated, drying the leaves and the remaining portion of the plant separately in an oven to obtain constant weight (for 48 h at 65 °C). The area of the green leaves taken from the destructive samples was recorded by leaf area meter (Model No: WDY- 500 A, Swastik Scientific Company, India). The ratio of the leaf area weight of these leaves was used to measure the LAI (Equation (1)) [28]. In the case of yield attributes, ten plants from a plot were randomly marked and at crop maturity; these were harvested, dried, and data on the yield parameters were noted.

$$\text{Leaf area index} = \frac{\text{leaf area}}{\text{ground area}} \quad (1)$$

2.4.2. Collection and Analysis of Plant and Soil Samples

N, P, K, S, and Zn content in plant samples was determined by the standard procedures (Table 1). Plant samples required for the determination of P, K, S and Zn were taken treatment wise after noting down the yields data and dried at 65 °C, pulverized, and digested in di-acid (9:4 v/v) of nitric acid (HNO_3)/perchloric acid ($HClO_4$). The nutrient content in straw and grain of rice was measured and nutrient uptake was determined by multiplying the nutrient content with the corresponding straw and grain yield [21].

$$\text{Nutrient uptake}\left(\text{kg ha}^{-1}\right) = \frac{\%\ \text{nutrient content in grain or straw} \times \text{dry matter}}{100} \quad (2)$$

Initial soil sample (0–15 cm) was collected prior to cultivation of *Kharif* rice in June 2014 and it was considered for determination of soil characteristics and initial fertility. After each harvest again soil samples were collected treatment wise to obtain the post-harvest soil nutrient status and it was further considered as the initial soil fertility for the next crop. Likewise, the final soil samples were collected in May 2016 after the harvest of *boro* rice. Collected soil samples were air-dried and ground to pass through a 2-mm stainless steel sieve for determination of soil parameters by standard methods as mentioned in Table 1. The initial soil fertility has also been mentioned in Table 1, however, the nutrient balance has been calculated crop-wise as well as for the system.

2.4.3. Nutrient Balance

The balance sheet of available nutrients was computed by using the following formulae given by Tandon [29] (Equation (3)). The determined nutrient balance may be positive or negative.

$$\text{Nutrient balance}\left(\text{kg ha}^{-1}\right) = \text{Available soil nutrient status} - \text{Initial soil status before each crop} \quad (3)$$

2.5. Calculations and Statistical Analysis

The experimental data were analysed statistically by using analysis of variance (ANOVA). The standard error of the mean (SEm±) and critical difference at 5% probability level of significance (CD, $p \leq 0.05$) [30] were calculated. The software used in the statistical analysis and drawing figures (including regression curve) was Excel from Microsoft Office Home and Student version 2019-en-us, Microsoft Inc., Redmond, Washington (DC, USA).

3. Results and Discussion

3.1. Growth Parameters

Different growth characteristics were calculated for the different periods of the rice–rice cropping system for two consecutive years. The data on plant height (Table 3) revealed that application of an ample dose of nutrients in *Kharif* rice (i.e., T_1: $N_{80}P_{40}K_{40}Zn_{25}S_{20}$) triggered a significant increase in height of the rice plants over the control (i.e., T_{12}: no fertilizer) at different days after transplanting (DAT) in both years. The treatment $N_{80}P_{40}K_{40}Zn_{25}S_{20}$ (T_1) produced the tallest rice plants in both years, while the shortest plant was observed in the control plots. However, the treatments T_2, T_4, T_6, T_8, and T_{10} were statistically on par with the enhancement of plant height in both years.

Table 3. Effect of nutrient management on the plant height (cm) of *Kharif* and *Boro* rice at different growth stages.

Treatments	Plant Height (cm)											
	20 DAT		40 DAT		60 DAT		80 DAT		100 DAT		120 DAT	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
***Kharif* rice**												
T_1	46.3 a	49.9 a	61.7 a	64.8 a	98.0 a	99.5 a	118.0 a	119.4 a	118.9 a	119.6 a	119.7 a	119.5 a
T_2	41.9 ab	46.2 a	59.2 ab	62.4 a	84.9 abc	91.4 ab	108.9 a	111.8 a	110.0 abc	111.9 abc	110.2 a	112.0 a–e
T_3	29.2 cd	26.1 b	40.0 cd	38.4 e	74.5 cd	70.0 c	94.5 a	92.9 a	96.1 bc	93.8 bc	96.8 a	94.0 de
T_4	44.1 a	44.8 a	60.1 ab	60.1 ab	95.0 ab	95.9 ab	115.0 a	116.1 a	116.6 a	116.6 ab	116.8 a	117.2 ab
T_5	38.7 ab	39.4 ab	48.3 bcd	49.2 d	79.9 bcd	80.9 ac	95.9 a	97.6 a	96.2 c	98.6 abc	98.5 a	98.9 cde
T_6	45.3 a	45.4 a	57.9 ab	60.1 ab	94.3 ab	93.6 ab	113.3 a	114.1 a	114.5 ab	114.4 ab	114.7 a	115.4 ab
T_7	41.6 ab	40.2 a	51.8 abc	52.0 bcd	83.0 a–d	81.2 bc	96.3 a	90.8 a	96.4 bc	91.3 bc	96.6 a	92.5 b–e
T_8	43.8 ab	44.4 a	57.1 ab	59.0 abc	91.9 ab	94.7 ab	111.9 a	113.9 a	111.8 abc	114.7 ab	111.7 a	116.6 abc
T_9	34.8 bcd	34.0 ab	50.8 a–d	51.2 bcd	82.5 a–d	81.1 bc	97.1 a	94.3 a	98.0 bc	95.5 abc	98.9 a	96.7 a–e
T_{10}	41.2 ab	45.6 a	59.2 ab	57.0 a–d	85.0 abc	95.0 ab	115.0 a	110.7 a	116.2 a	112.2 abc	116.3 a	112.1 a–d
T_{11}	37.4 abc	38.7 ab	51.9 abc	50.2 cd	80.7 acd	80.0 bc	98.7 a	93.0 a	98.1 bc	94.6 bc	98.1 a	93.8 a–e
T_{12}	26.1 d	25.6 b	38.3 d	34.5 e	68.3 d	67.0 c	88.3 b	86.3 b	88.5 c	87.5 c	88.6 a	87.6 e
F-test	**	**	**	**	**	**	*	*	**	**	*	**
SEm (±)	2.5	3.0	3.3	4.2	5.1	6.0	6.5	6.2	6.3	6.3	6.6	6.3
CV (%)	7.12	13.7	8.12	12.3	6.56	7.17	10.0	11.4	8.43	8.18	9.96	8.21
***Boro* rice**												
T_1	51.3 a	52.9 a	82.0 a	84.2 a	104.1 a	106.2 a	121.0 a	124.1 a	122.0 a	124.0 a	122.0 a	124.8 a
T_2	46.9 a	48.6 ab	72.3 abc	76.4 abc	103.8 a	104.7 a	116.2 ab	117.4 abc	116.0 ab	118.0 abc	117.0 a	119.0 a
T_3	34.1 bc	33.1 c	56.3 de	52.6 e	78.3 b	74.4 b	97.4 a–e	94.6 bcd	98.4 abc	94.3 cd	98.4 ab	94.4 ab
T_4	49.1 a	50.1 ab	74.3 ab	84.9 a	103.6 a	104.4 a	118.9 a	116.1 abc	118.0 ab	117.0 abc	118.0 a	116.8 ab
T_5	44.6 abc	45.2 ab	68.5 bcd	68.3 cd	80.0 b	78.8 b	91.9 cde	93.0 cd	95.4 bc	94.2 cd	95.2 ab	94.5 ab
T_6	47.1 a	47.9 ab	75.1 ab	81.2 a	102.9 a	103.7 a	117.1 ab	117.6 abc	119.0 ab	119.0 ab	120.0 a	118.9 a
T_7	45.1 abc	44.2 abc	69.1 bc	69.0 bcd	84.4 ab	85.4 ab	89.9 de	97.1 bcd	99.1 abc	97.6 bcd	99.3 ab	98.1 ab
T_8	48.8 a	49.4 ab	70.3 abc	78.7 ab	103.1 a	104.3 a	115.1 abc	123.3 a	118.0 ab	125.0 a	118.0 a	124.7 a
T_9	41.8 abc	42.4 abc	70.0 abc	68.1 cd	86.4 ab	78.3 b	91.5 cde	98.2 bcd	101.0 abc	98.6 abc	101.2 ab	98.9 ab
T_{10}	46.2 ab	47.9 ab	75.3 ab	83.7 a	104.5 a	105.3 a	112.3 a–d	119.4 ab	116.0 ab	119.0 ab	115.0 ab	119.2 a
T_{11}	40.2 abc	40.8 bc	60.7 cde	64.0 d	86.9 ab	87.1 ab	93.4 b–e	94.5 cd	100.0 abc	97.9 bcd	100.1 ab	98.2 ab
T_{12}	33.1 c	32.3 c	54.0 e	51.0 e	71.9 b	69.7 b	83.0 e	80.2 d	86.5 c	81.6 d	87.0 b	81.7 b
F-test	**	**	**	**	**	**	**	**	**	**	**	**
SEm (±)	1.8	2.0	4.1	5.0	5.6	6.4	7.5	7.5	6.5	8.8	6.8	7.5
CV (%)	9.26	9.14	6.26	4.83	7.50	7.77	7.77	7.85	8.09	7.62	9.27	11.5

***Kharif* season:** T_1: $N_{80}P_{40}K_{40}Zn_{25}S_{20}$; T_2: $N_{40}P_{40}K_{40}Zn_{25}S_{20}$; T_3: $N_0P_{40}K_{40}Zn_{25}S_{20}$; T_4: $N_{80}P_{20}K_{40}Zn_{25}S_{20}$;$T_5$: $N_{80}P_0K4_0Zn_{25}S_{20}$; T_6: $N_{80}P_{40}K_{20}Zn_{25}S_{20}$; T_7: $N_{80}P_{40}K_0Zn_{25}S_{20}$; T_8: $N_{80}P_{40}K_{40}Zn_{12.5}S_{20}$;$T_9$: $N_{80}P_{40}K_{40}Zn_0S_{20}$; T_{10}: $N_{80}P_{40}K_{40}Zn_{25}S_{10}$; T_{11}: $N_{80}P_{40}K_{40}Zn_{25}S_0$ and T_{12}: control; (without any fertilizer); ***Boro* season:** T_1: $N_{120}P_{60}K_{60}Zn_{25}S_{20}$; T_2: $N_{60}K_{60}Zn_{25}S_{20}$; T_3: $N_0K_{60}Zn_{25}S_{20}$; T_4: $N_{120}P_{30}K_{60}Zn_{25}S_{20}$; T_5: $N_{120}P_0K_{60}Zn_{25}S_{20}$; T_6: $N_{120}P_{60}K_{30}Zn_{25}S_{20}$; T_7: $N_{120}P_{60}K_0Zn_{25}S_{20}$; T_8: $N_{120}P_{60}K_{60}Zn_{12.5}S_{20}$; T_9: $N_{120}P_{60}K_{60}Zn_0S_{20}$; T_{10}: $N_{120}P_{60}K_{60}Zn_{25}S_{10}$; T_{11}: $N_{120}P_{60}K_{60}Zn_{25}S_0$ and T_{12}: control (without any fertilizer). CV (%) = coefficient of variation; ** and * significant at $p \leq 0.01$ and $p \leq 0.05$, respectively; NS = not significant; different letters within the continuous columns indicate significant differences at the 1% level of probability.

In the case of *Boro* rice, the application of T_1 also produced the longest plants at all growth stages amongst all other treatments during the two years of study (2014–2015 and 2015–2016). The observation clearly showed that the application of 100% recommended dose of N:P:K:Zn:S (also termed as ample dose) increased the plant height at the different growth stages of rice irrespective of seasons, probably because of the proper nutrition obtained by the said treatment. Similar findings were also noted by earlier researchers [31], who also revealed that the application of balanced nutrients in a crop improved the growth and development of plants.

Dry matter accumulation of the *Kharif* and *Boro* rice in both years (2014 and 2015) was influenced by different levels of nutrients and there was an enhancement in dry matter with the progression of crops towards maturity (Table 4). A strong interrelationship between dry matter accumulation and yield was observed (R_2 being 0.83 and 0.89, respectively, for the *Kharif* and *Boro* seasons). The treatment T_1 in both the *Kharif* and *Boro* seasons resulted in the production of the maximum dry matter at all growth stages. The treatment T_1 in *Kharif* rice increased the dry matter production significantly in both seasons over T_3, T_5, T_7, T_9, T_{11}, and the control, but the treatment T_1 was statistically on par with T_2, T_4, T_6, T_8, and T_{10}.

A similarity between the two years was noted in *Boro* rice where T_1 expressed its significant superiority over the control (T_{12}, no fertilizer) as observed in different growth stages and T_3 was statistically on par with control at the harvesting stage during both years of study. The ample dose of nutrients (T_1) produced significantly more dry matter than T_3, T_5, T_7, T_9, T_{11}, and the control. Although, treatment T_1 was statistically on par with the T_2, T_4, T_6, and T_{10} treatments in both seasons. The maximum dry matter in the T_1 treatment was due to the application of 100% of the recommended dose of N:P:K:Zn:S that facilitated access to the required nutrients involving in dry matter production; this assumption was also confirmed by earlier studies [32,33].

The data on *Kharif* and *Boro* rice for LAI was measured at different growth stages, where an ample dose of nutrients enhanced the LAI over the control (T_{12}, no fertilizer); although, an improvement in the LAI did not differ significantly in all the growth stages in both years (Table 5). The LAI value of rice gradually increased for the *Kharif* and *Boro* rice during both the years for all treatments and reached its maximum values at 60 DAT, followed by a decline as the crops reached maturity. In the case of *Kharif* rice, the maximum LAI was noted at 60 DAT with T_1 and it was statistically on par with all treatments in 2014 and 2015. Similar to *Kharif* rice, the LAI of *Boro* rice at different growth stages in both years also did not differ significantly for all treatments. Among these treatments, the higher value of LAI was recorded in the T_1 treatment and the minimum LAI value was in the control plots; although, the LAI for all treatments did not differ significantly. Considering the growth stages, the higher LAI was observed at 60 DAT in both years and the minimum value was at 100 DAT (Table 5). The application of the recommended dose of nutrients produced a higher LAI value, due to the proper nutrition in the plant helping it attaining sufficient vegetative growth (LAI) and keeping the crop healthy irrespective of growth stages and year. This assumption was also confirmed by several earlier studies [34,35], who also revealed an increase in LAI with the recommended dose of N:P:K:Zn:S.

The number of tillers m^{-2} of *Kharif* and *Boro* rice was also influenced by nutrient management, observed during two consecutive years (Table 6). Application of an ample dose of nutrients, i.e., $N_{80}P_{40}K_{40}Zn_{25}S_{20}$ in *Kharif* rice and $N_{120}P_{60}K_{60}Zn_{25}S_{20}$ in *Boro* rice, resulted in the production of the maximum number of tillers over the control at all the growth stages. Considering the growth stages, tillers m^{-2} of the *Kharif* rice at 20 DAT were significant in both years, and 40 and 60 DAT only for all treatments; although, the maximum tillers m^{-2} was recorded in T_1 and the lowest was in the T_{12} (control) treatment. With little exception, tillers m^{-2} for T_1 was statistically on par with T_2, T_4, T_6, T_8, and T_{10} in increasing the number of tillers during both years; however, T_1 significantly produced more tillers than T_3, T_5, T_7, T9, T_{11}, and T_{12} (control).

Table 4. Effect of nutrient management on dry matter accumulation (g) of *Kharif* and *Boro* rice at different growth stages.

Treatment	Dry Matter Accumulation (g m^{-2})											
	20 DAT		40 DAT		60 DAT		80 DAT		100 DAT		120 DAT	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
						Kharif rice						
T_1	156.1 a	158.2 a	416.0 a	420.9 a	760.3 a	768.0 a	1170.0 a	1181.5 a	1280.0 a	1290.9 a	1301.1 a	1315.5 a
T_2	150.8 a	153.0 a	390.1 ab	409.5 a	728.9 ab	750.3 a	1134.4 abc	1137.6 a	1240.6 a	1243.8 a	1247.6 a	1257.8 a
T_3	140.8 a	140.3 a	305.9 bc	302.3 ab	510.1 cd	518.9 bc	602.3 d	606.9 c	657.6 b	658.3 d	660.6 bc	662.8 d
T_4	153.6 a	153.8 a	404.0 a	407.3 a	735.4 ab	750.5 a	1132.1 abc	1138.7 a	1225.9 a	1236.6 a	1235.9 a	1249.2 ab
T_5	141.0 a	140.8 a	370.4 ab	373.8 ab	506.7 cd	507.8 bc	900.0 c	906.3 b	1002.9 a	1006.9 c	1009.9 ab	1016.4 c
T_6	155.2 a	157.3 a	407.3 a	409.9 a	745.6 ab	750.7 a	1140.9 ab	1140.0 a	1222.8 a	1233.0 a	1230.8 a	1235.6 ab
T_7	140.7 a	142.9 a	373.9 ab	357.3 ab	521.9 cd	505.2 bc	903.3 c	902.2 b	1000.4 a	1008.8 c	1006.4 ab	1012.4 c
T_8	155.0 a	159.2 a	406.9 a	410.3 a	742.9 ab	750.5 a	1110.8 abc	1137.6 a	1209.8 a	1225.6 a	1229.8 a	1245.6 ab
T_9	140.4 a	147.6 a	374.2 ab	380.6 ab	506.6 cd	508.3 bc	909.8 bc	908.2 b	1002.1 a	1005.3 c	1003.1 ab	1009.7 c
T_{10}	155.7 a	156.5 a	403.5 a	404.8 a	745.5 ab	749.5 a	1108.3 abc	1111.7 a	1210.1 a	1215.9 ab	1220.1 a	1225.5 ab
T_{11}	140.0 a	147.1 a	363.0 ab	370.5 ab	590.0 bc	609.7 ab	950.3 abc	952.7 b	1040.3 a	1040.5 bc	1042.3 a	1045.7 bc
T_{12}	101.7 b	100.5 b	260.0 c	255.3 b	408.8 d	407.5 c	508.3 d	505.3 c	535.2 b	532.3 d	518.0 c	514.5 da
F-test	*	*	**	**	**	**	**	**	*	**	**	**
SEm (±)	5.1	3.6	13.7	12.6	33.4	26.4	49.7	47.4	52.3	61.5	57.0	72.3
CV (%)	7.21	5.92	7.96	13.29	8.86	10.04	8.25	4.63	10.30	5.86	11.74	6.43
						Boro rice						
T_1	177.1 a	180.0 a	437.0 a	452.5 a	870.3 a	885.7 a	1370.0 a	1380.7 a	1507.2 a	1511.1 a	1519.3 a	1515.1 a
T_2	175.8 ab	175.4 a	411.1 a	415.8 ab	830.9 a	831.6 a	1300.4 a	1307.0 a	1457.7 a	1408.6 a	1424.7 a	1410.6 a
T_3	155.8 a	154.5 ab	308.9 a	304.6 c	421.1 b	410.5 b	656.3 b	652.6 b	758.4 b	768.6 b	759.2 b	766.7 b
T_4	172.6 ab	168.7 a	425.0 a	426.6 a	846.4 a	847.5 a	1333.1 a	1331.4 a	1492.9 a	1451.8 a	1454.0 a	1451.8 a
T_5	160.0 ab	155.9 ab	383.4 a	387.6 abc	732.7 a	728.2 a	1209.0 a	1215.2 a	1309.5 a	1313.9 a	1325.0 a	1313.9 a
T_6	186.2 a	172.2 a	428.3 a	425.6 ab	849.6 a	848.6 a	1335.9 a	1342.9 a	1493.3 a	1452.8 a	1453.7	1452.8 a
T_7	160.7 ab	153.3 ab	384.9 a	380.6 abc	720.9 a	730.5 a	1208.3 a	1202.8 a	1300.6 a	1300.3 a	1320.9 a	1320.3 a
T_8	176.0 a	173.1 a	417.9 a	441.1 a	840.8 a	845.2 a	1331.8 a	1325.2 a	1491.0 a	1450.8 a	1451.2 a	1450.8 a
T_9	160.4 ab	154.0 ab	389.2 a	383.4 abc	721.6 a	753.4 a	1207.8 a	1202.5 a	1303.5 a	1300.1 a	1306.9 a	1310.1 a
T_{10}	176.7 a	178.1 a	424.5 a	449.1 a	846.5 a	847.7 a	1329.3 a	1331.0 a	1495.0 a	1452.1 a	1457.8 a	1452.1 a
T_{11}	161.0 ab	150.6 ab	389.8 a	384.9 abc	719.0 a	755.9 a	1209.3 a	1205.5 a	1302.7 a	1300.3 a	1325.0 a	1315.3 a
T_{12}	132.7 b	130.1 b	321.0 a	317.0 bc	450.8 b	445.6 b	564.3 b	560.3 b	587.0 b	591.8 b	582.1 b	591.8 b
F-test	*	*	*	**	*	*	*	*	*	*	*	*
SEm (±)	5.2	4.8	9.6	13.0	37.3	39.8	51.6	55.7	62.2	56.3	62.5	59.3
CV (%)	6.83	7.75	14.10	9.21	7.79	8.05	7.20	5.47	8.35	7.12	8.14	8.31

Treatment details of the *Kharif* and *Boro* rice are mentioned in Table 3. CV (%) = coefficient of variation; ** and * significant at $p \leq 0.01$ and $p \leq 0.05$, respectively; NS = not significant; different letters within the continuous columns indicate significant differences at the 1% level of probability.

Table 5. Effect of nutrient management on the leaf area index of rice at different growth stages.

Treatment	Leaf Area Index (LAI)									
	20 DAT		40 DAT		60 DAT		80 DAT		100 DAT	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Kharif rice										
T_1	2.66	2.66	3.07	3.09	4.87	4.88	2.71	2.70	1.44	1.33
T_2	2.66	2.64	3.05	3.04	4.86	4.85	2.69	2.68	1.42	1.30
T_3	1.72	1.39	2.11	1.84	3.85	3.82	1.67	1.71	0.43	0.29
T_4	2.65	2.62	3.04	3.05	4.85	4.86	2.70	2.70	1.40	1.30
T_5	2.61	2.61	3.00	2.99	4.83	4.84	2.70	2.66	1.33	1.22
T_6	2.64	2.62	3.05	3.01	4.86	4.87	2.70	2.69	1.42	1.24
T_7	2.58	2.59	2.98	3.01	4.82	4.84	2.64	2.66	1.32	1.18
T_8	2.62	2.61	3.02	2.99	4.87	4.88	2.64	2.71	1.40	1.20
T_9	1.76	1.76	2.17	2.19	3.97	3.98	1.81	1.80	0.54	0.43
T_{10}	2.65	2.64	3.05	3.06	4.87	4.88	2.69	2.69	1.42	1.27
T_{11}	2.56	2.54	2.97	2.97	4.85	4.87	2.67	2.70	1.36	1.24
T_{12}	1.61	1.36	1.95	1.74	3.52	3.56	1.35	1.38	0.20	0.05
F-test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm (±)	0.04	0.07	0.06	0.08	0.08	0.1	0.07	0.08	0.03	0.03
CV (%)	45.49	46.78	39.11	39.82	23.79	23.79	0.2	45.02	86.57	84.88
Boro rice										
T_1	2.83	2.74	3.38	3.45	5.54	5.53	3.43	3.40	2.91	2.95
T_2	2.68	2.70	3.40	3.42	5.51	5.50	3.40	3.41	2.89	2.91
T_3	1.70	1.66	2.45	2.41	4.48	4.41	2.38	2.43	1.91	1.89
T_4	2.70	2.68	3.40	3.44	5.51	5.45	3.42	3.44	2.80	2.91
T_5	2.60	2.61	3.34	3.37	5.51	5.43	3.41	3.46	2.77	2.88
T_6	2.74	2.72	3.44	3.45	5.52	5.48	3.41	3.47	2.78	2.88
T_7	2.68	2.69	3.40	3.40	5.43	5.48	3.36	3.38	2.78	2.79
T_8	2.74	2.76	3.44	3.45	5.44	5.48	3.36	3.57	2.89	2.88
T_9	1.90	1.84	2.48	2.55	4.64	4.63	2.53	2.50	2.01	2.05
T_{10}	2.70	2.71	3.42	3.42	5.50	5.49	3.40	3.45	2.83	2.89
T_{11}	2.69	2.70	3.41	3.44	5.45	5.47	3.38	3.43	2.77	2.84
T_{12}	1.59	1.56	2.36	2.33	4.14	4.04	1.96	1.94	1.54	1.52
F-test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm (±)	0.06	0.05	0.04	0.04	0.1	0.1	0.06	0.07	0.09	0.08
CV (%)	44.41	44.41	34.66	34.39	20.94	21.01	34.94	34.54	42.53	41.72

The treatment details for *Kharif* and *Boro* rice are mentioned in Table 3. CV (%) = coefficient of variation; NS = not significant.

Table 6. Effect of nutrient management on tillers (m^{-2}) of rice at different growth stages.

Treat	Tillers (m^{-2})											
	20 DAT		40 DAT		60 DAT		80 DAT		100 DAT		120 DAT	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
						Kharif rice						
T_1	298.8 a	299.7 a	311.2	315.8 a	340.3	340.7 a	327.8 a	326.8	324.7 a	310.4	317.3 a	306.8
T_2	296.9 a	292.1 a	300.3	308.8 a	330.2	333.3 a	305.3 ab	312	303.6 ab	298.3	298.6 ab	295.2
T_3	263.1 a	228.6 a	278.8	274.1 ab	280.2	295.7 a	270.5 ab	286.3	263.1 ab	259.4	257.8 ab	259.1
T_4	289.1 a	290.8 a	290.3	298.1 a	331.7	333.3 a	302.9 ab	312.7	302.5 ab	294.3	300.2 ab	291.6
T_5	263.6 a	265.2 a	280.1	284.9 ab	280.3	288.5 ab	281.2 ab	280.2	280.9 ab	275.7	280.3 ab	274.0
T_6	290.1 a	292.2 a	296.3	301.1 a	322.6	330.0 a	319.2 a	307.8	313.1 a	301.2	310.2 a	296.4
T_7	265.0 a	264.0 a	280.5	282.2 ab	282.3	283.7 ab	280.7 ab	299.8	278.9 ab	281.2	271.5 ab	277.4
T_8	291.8 a	293.3 a	296.3	306.2 a	337.3	340.1 a	318.7 a	320.2	312.1 a	297.7	309.3 a	296.0
T_9	263.3 a	267.0 a	273.2	283.5 ab	280.8	287.0 ab	285.0 ab	302.5	280.5 ab	284.0	274.7 ab	274.1
T_{10}	287.3 a	293.7 a	290.1	294.2 ab	325.5	325.3 a	313.0 a	309.5	309.6 a	296.6	305.3 a	293.1
T_{11}	280.2 a	285.1 a	281.5	290.4 ab	281.9	301.7 a	282.0 ab	299.4	280.3 ab	276.3	275.2 ab	271.3
T_{12}	198.3 b	151.3 b	206.0	205.7 b	212.7	210.7 b	208.7 b	208.0	204.5 b	200.8	201.7 b	195.9
F-test	*	*	NS	*	NS	**	**	NS	**	NS	**	NS
SEm (±)	7.7	10.2	9.4	9.6	9.6	12.7	10.5	10.4	8.7	8.4	11.2	9.8
CV (%)	13.49	9.54	13.65	10.53	15.50	9.31	11.84	18.94	11.98	23.91	12.16	12.42
						Boro rice						
T_1	340.8 a	346.8 a	361.2	362.7 a	386.3 a	388.3 a	380.8 a	365.7	366.7 a	366.0	361.3 a	360.3 a
T_2	326.9 a	325.1 a	330.3	335.1 a	383.6 a	383.8 a	375.3 a	372.3	368.6 a	363.0	360.0 a	361.8 a
T_3	293.1 a	308.6 a	310.8	316.3 a	342.0 ab	340.3 ab	330.5 ab	331.7	321.1 a	325.7	300.8 ab	320.3 ab
T_4	332.1 a	325.8 a	339.3	336.7 a	371.7 a	373.6 a	362.5 a	367.3	359.9 a	366.0	357.2 a	353.0 a
T_5	299.6 a	323.0 a	330.1	330.3 a	345.3 ab	337.4 ab	331.2 ab	328.5	326.9 a	323.5	320.3 ab	320.3 ab
T_6	337.1 a	332.2 a	346.3	348.3 a	362.6 a	372.9 a	359.2 a	370.0	353.1 a	369.2	347.2 a	362.5 a
T_7	303.0 a	320.0 a	331.0	330.7 a	350.3 ab	343.3 a	330.7 ab	330.7	325.9 a	326.5	321.5 ab	323.3 ab
T_8	338.8 a	333.3 a	340.3	341.3 a	383.3 a	373.1 a	358.7 a	363.6	352.1 a	361.3	350.3 a	352.7 a
T_9	303.3 a	307.0 a	313.2	322.2 a	342.0 ab	346.3 ab	338.6 ab	332.0	331.5 a	326.5	321.7 ab	323.5 ab
T_{10}	327.3 a	333.7 a	328.1	330.3 a	365.5 a	373.5 a	353.0 a	365.3	350.6 a	363.5	345.3 a	356.9 a
T_{11}	309.2 a	319.0 a	323.5	325.0 a	349.9 ab	345.9 ab	342.0 a	330.7	335.3 a	322.2	315.2 ab	320.3 ab
T_{12}	208.3 b	211.3 b	226.0	221.7 b	252.7 b	257.3 b	245.7 b	240.7	233.5 b	228.0	229.7 b	223.3 b
F-test	*	*	NS	*	**	**	**	NS	*	NS	**	**
SEm (±)	10.2	11.3	9.9	10.4	12.2	13.9	12	11.4	10.2	10	13.2	10.8
CV (%)	7.46	8.12	16.50	9.30	9.44	10.90	9.37	17.68	8.56	22.52	9.83	12.30

The treatment details for the *Kharif* and *Boro* rice are mentioned in Table 3. CV (%) = coefficient of variation; ** and * significant at $p \leq 0.01$ and $p \leq 0.05$, respectively; NS = not significant; different letters within the continuous columns indicate significant differences at the 1% level of probability.

A similar trend was also noted in *Boro* rice, where an ample dose of recommended nutrients (T_1) produced maximum tillers per unit area. Treatment T_1 showed its significant superiority to T_3, T_5, T_7, T_9, T_{11}, and the control (T_{12}, no fertilizer) during both years, but T_1 remained statistically on par with T_2, T_4, T_6, T_8, and T_{10}. Omission of all nutrients in T_{12} was totally dependent on inherent soil fertility and, due to lack of sufficient nutrients, it did not produce the desired number of tillers. On the other hand, the treatment T_1 received an ample dose of recommended nutrients that facilitated proper nutrition and resulted in maximum tillers per unit area at different growth stages of *Kharif* and *Boro* rice during both the years of study. The beneficial effects of fertilizers in enhancing tillers were earlier observed by researchers [36,37].

3.2. Yield Attributes and Yield

Yield attributes such as panicles m^{-2}, grains panicle^{-1}, spikelets panicle^{-1}, test weight, and panicle length were recorded for both *Kharif* and *Boro* rice during both seasons (Table 7). Considering both seasons data of these parameters, panicles m^{-2}, grains panicle^{-1}, spikelets panicle^{-1}, and test weight varied significantly only in the first season. The recommended dose of nutrients (T_1) registered higher values than T_3, T_5, T_7, T_9, T_{11}, and T_{12} (control) in both years. However, the treatment with an ample dose of nutrients (T_1) was statistically on par in increasing the values of the yield attributes.

The yield-attributing characters of *Boro* rice did not differ significantly in both years, where T_1 exerted higher values over T_3, T_5, T_7, T_9, T_{11}, and the control (T_{12}, no fertilizer) during 2014–2015 and 2015–2016. However, T_1 was statistically on par with the T_2, T_4, T_6, T_8, and T_{10} treatments. The results corroborate the findings of earlier studies [34,35], where researchers revealed that balanced doses of all nutrients influence the proper growth and development of plants, leading to improved yield-attributing characters of rice.

The 'R' values of the yield attributes were reflected in the productivity of the *Kharif* and *Boro* rice in terms of grain and straw yields during both the years of study (Figures 2–7). The data showed that the grain yield of *Kharif* rice was at its maximum (5.46 and 5.67 t ha^{-1} in 2014 and 2015, respectively) with the treatment T_1 (Figure 8). In 2014, the treatment with an ample dose of nutrients (T_1) produced significantly more grain yield than T_2, T_3, and the control (T_{12}); but, in 2015, T_1 yielded significantly more than T_3 and the control. The other treatments were statistically on par with T_1. The grain yield of *Boro* rice was higher with the application of an ample dose of the recommended fertilizer (T_1) that yielded 6.6 t ha^{-1} in both years. The treatments were statistically on par with the other treatments, except for T_2, T_3, and T_{12} (unfertilized control), in increasing the grain yield of *Boro* rice during both years. In the rice–rice cropping system also, both the *Kharif* and *Boro* rice yielded more with the ample dose of nutrients application. A similar type of impact of an ample dose of recommended nutrients was earlier noted by Mohapatra [38] and Trivedi et al. [39].

Table 7. Effect of nutrient management on the yield attributes of rice.

Treatments	Yield Attributes of Rice									
	Panicles m^{-2}		Grains $Panicle^{-1}$		Spikelets $Panicle^{-1}$		Test Weight (g)		Panicle Length (cm)	
	2014	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
					Kharif rice					
T_1	246.1 a	249.8	131.0 a	133.1	148.3 a	151.0	23.5 a	24.2	30.0	31.2
T_2	241.1 b	244.8	127.0 b	128.2	146.5 ab	146.3	22.6 a	22.8	28.7	29.7
T_3	200.2 f	219.7	110.0 d	110.5	133.0 ef	132.1	22.5 a	22.3	26.0	22.0
T_4	245.0 a	245.9	120.0 c	124.5	143.0 c	148.0	22.8 a	23.2	29.9	30.9
T_5	221.1 b	220.2	111.5 d	112.5	135.1 de	135.5	22.2 a	22.6	26.4	26.8
T_6	232.7 c	236.5	125.2 b	128.6	147.5 ab	150.9	21.8 a	23.8	28.2	29.9
T_7	220.2 d	221.0	112.1 d	114.8	136.5 d	135.1	22.6 a	23.6	26.3	26.1
T_8	245.0 a	247.0	122.2 c	127.6	147.3 ab	148.8	22.5 a	23.2	29.3	30.6
T_9	220.2 d	214.2	112.1 d	113.2	136.2 d	135.0	22.2 a	23.5	26.2	26.7
T_{10}	245.0 a	245.1	111.0 d	125.4	144.9 bc	146.1	22.2 a	23.2	27.0	30.4
T_{11}	220.6 d	220.2	112.1 d	114.0	131.3 fg	136.0	22.2 a	23.0	25.0	25.7
T_{12}	212.3 e	204.0	65.5 e	61.8	129.7 g	127.1	20.0 b	19.7	21.0	19.1
F-test	**	NS	**	NS	**	NS	*	NS	NS	NS
SEm (±)	8.8	9.8	6.4	6.1	3.9	5.1	1.7	1	1.1	1.4
CV (%)	24.9	28.7	18.8	17.8	11.4	14.9	4.9	2.9	3.4	4.1
					Boro rice					
T_1	281.0	282.2	140.0	145.4	155.1	159.4	24.0	25.6	32.5	33.2
T_2	269.7	280.4	133.0	136.0	151.0	152.2	22.9	22.8	29.9	30.3
T_3	239.8	234.7	90.0	91.5	126.2	125.1	21.0	20.3	26.6	25.3
T_4	260.4	274.3	125.0	140.1	154.9	156.1	23.3	23.5	31.4	33.1
T_5	233.9	236.4	121.2	120.0	131.3	136.0	22.7	22.6	26.4	27.1
T_6	277.6	279.5	130.0	132.3	155.0	158.0	22.3	23.8	30.1	32.3
T_7	232.5	237.7	120.0	121.1	130.4	131.7	23.1	23.4	29.0	28.7
T_8	278.0	281.4	134.9	136.3	150.6	153.0	23.0	23.2	31.5	32.1
T_9	232.0	235.2	120.0	122.0	134.4	137.9	23.8	24.9	22.4	24.1
T_{10}	267.0	268.1	137.9	138.2	151.1	155.3	22.6	23.6	25.5	27.2
T_{11}	236.6	230.2	121.6	121.2	130.7	131.0	22.0	23.1	24.9	26.6
T_{12}	215.3	210.0	80.0	76.0	121.0	120.0	20.2	20.0	22.8	20.3
F-test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm (±)	8	8.5	6.2	6.9	6	5.9	1.8	1	1	1.4
CV (%)	23.4	25.1	18.1	20.1	17.6	17.3	5.2	2.8	2.8	4.0

The treatment details for *Kharif* and *Boro* rice are mentioned in Table 3. CV (%) = coefficient of variation; ** and * significant at $p \leq 0.01$ and $p \leq 0.05$, respectively; NS = not significant; different letters within the continuous columns indicate significant differences at the 1% level of probability.

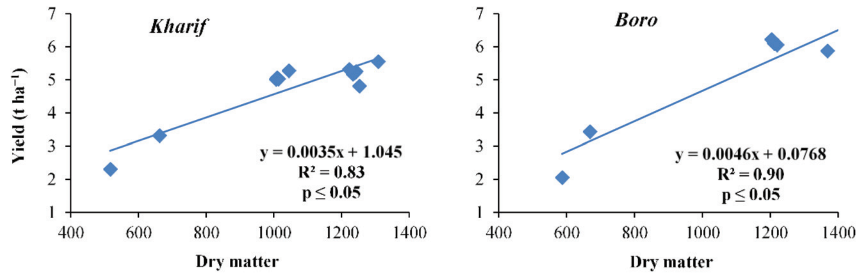

Figure 2. Relation between dry matter and yield of *Kharif* and *Boro* rice.

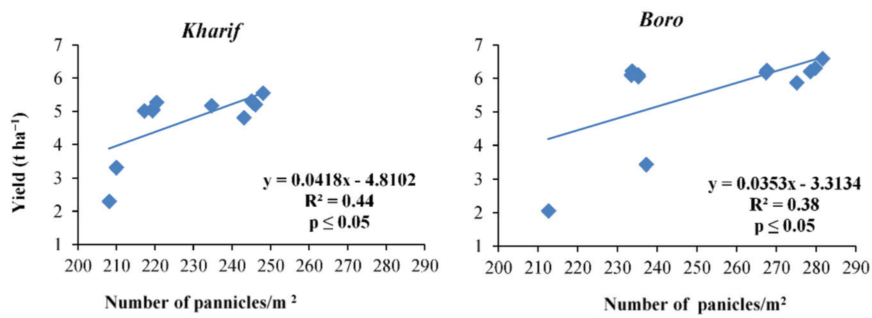

Figure 3. Relation between number of panicles m^{-2} and yield of *Kharif* and *Boro* rice.

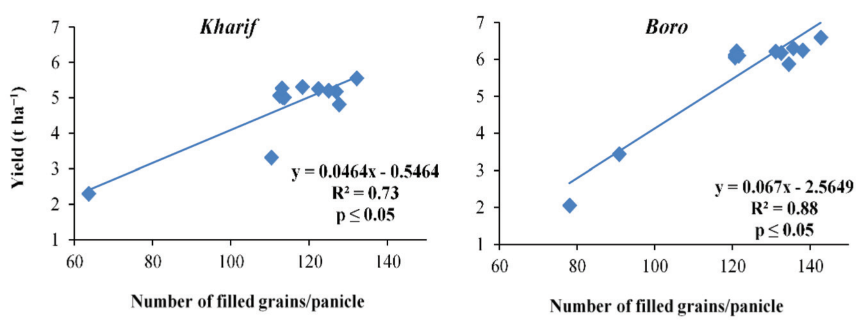

Figure 4. Relation between number of filled grains $panicle^{-1}$ and yield of *Kharif* and *Boro* rice.

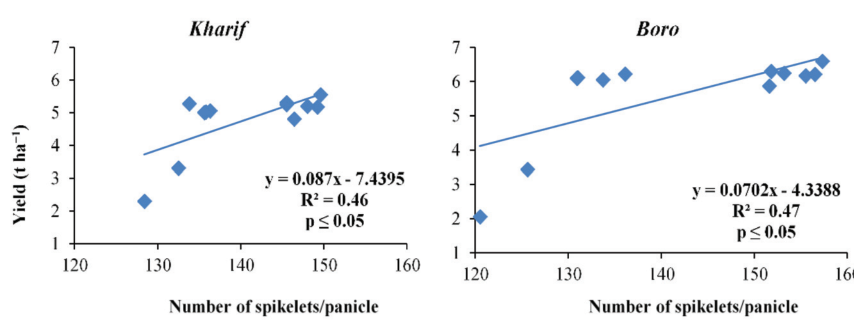

Figure 5. Relation between number of spikelets $panicle^{-1}$ and yield of *Kharif* and *Boro* rice.

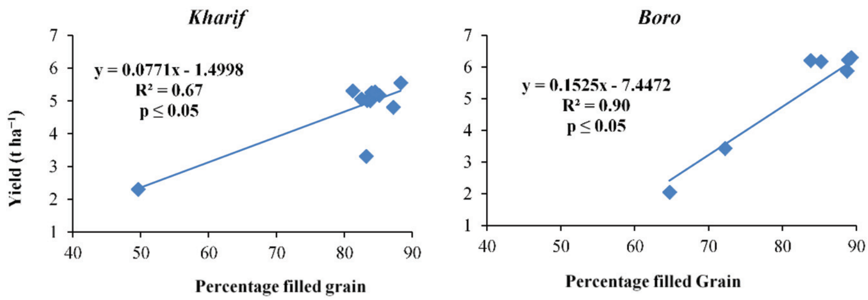

Figure 6. Relation between percentages filled grain and yield of *Kharif* and *Boro* rice.

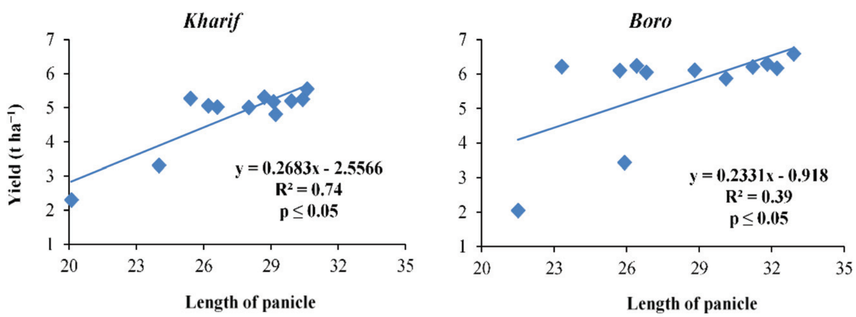

Figure 7. Relation between length of panicle and yield of *Kharif* and *Boro* rice.

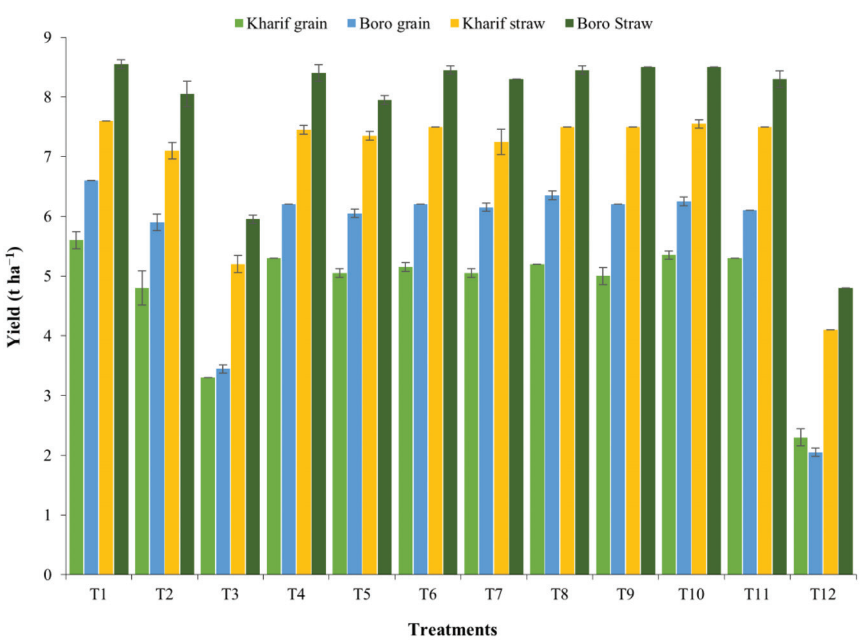

Figure 8. Effect of nutrient management on the yield of *Kharif* rice. SE± in each bar was calculated from three replications for every treatment.

Nutrient levels influenced straw yield in the rice–rice cropping system as noted in the case of grain yield. The treatment comprising recommended dose of N+P+K+S+Zn resulted in significant improvement in straw yield of *Kharif* and *Boro* rice over unfertilized control treatments during both the years of experimentation (Figure 8). In *Kharif* rice, the treatment T_1 produced the maximum straw yield and it was significantly more than T_3 and T_{12} (unfertilized control) in 2014, but in 2015, the application of T_1, being statistically on par with the other nutrient management treatments, significantly registered more straw yield over the control (T_{12}). The straw yield of *Boro* rice was maximum with T_1 and it remained significantly more than T_3 and T_{12} (control) during both 2014–2015 and 2015–2016. As the ample dose of nutrient application produced more dry matter than the control, grain and straw yields also followed a similar trend because the maximum biomass production was reflected with said treatment. The results conform with the findings of Trivedi et al. [39], who also noted higher biomass production with the recommended dose of nutrients in rice.

3.3. Nutrient Uptake

The uptake of N, P, K, S, and Zn by the grain and straw of *Kharif* and *Boro* rice were obtained by multiplying the grain and straw yield with the nutrient content of the grain and straw of the respective treatments. The results are presented below (Tables 8–10).

Table 8. Effect of nutrient management on the nutrient uptake (kg ha^{-1}) of *Kharif* rice.

Treatment	N Uptake in Grain		N Uptake in Straw		P Uptake in Grain		P Uptake in Straw		K Uptake in Grain	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
T_1	68.5	71.7	29.6	31.9	19.3	21.0	11.8	14.1	23.6	24.9
T_2	55.7	61.8	25.2	27.4	17.3	18.5	10.4	13.3	20.9	22.8
T_3	30.9	28.7	9.7	11	16.1	15.3	10.2	9.7	19.3	14.2
T_4	64.3	65.5	27.7	28.5	16.5	18.4	10.2	12.2	21.6	22.1
T_5	64.3	64.8	28.4	29.7	16.0	15.5	8.8	9.2	20.5	20.9
T_6	65.9	65.6	28.4	29.1	17.7	19.2	11.2	12.5	16.4	16.9
T_7	65.3	63.8	27.9	29.8	16.9	18.4	10.9	13.2	10.1	9.1
T_8	64.5	65.7	27.9	30.8	17.4	18.5	11.3	13.2	22.4	22.7
T_9	66.4	63.7	27.7	29.8	16.8	18.0	11.8	13.3	20.9	22.8
T_{10}	66.4	67	27.5	30.3	17.0	18.7	11.5	13.1	21.7	22.8
T_{11}	65.5	66.5	27.5	30.3	17.9	18.4	11.8	13.3	22.4	22.4
T_{12}	20.8	16.4	14.3	9.4	7.57	6.45	4.5	4.6	4.34	3.11
F-test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm (±)	1.33	2.26	0.83	0.83	0.8	0.83	0.49	0.48	1.2	0.9
CV (%)	3.91	6.63	2.44	2.43	2.50	2.40	1.40	1.41	3.5	2.65
Treatment	K uptake in straw		Zn uptake in grain		Zn uptake in straw		S uptake in grain		S uptake in straw	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
T_1	47.8	47.9	0.13	0.14	0.29	0.30	6.7	7.0	3.1	3.2
T_2	43.0	45.0	0.12	0.12	0.26	0.25	6.6	6.5	2.9	3.0
T_3	32.1	32.0	0.07	0.06	0.18	0.17	3.4	3.7	1.7	1.8
T_4	45.9	47.1	0.13	0.11	0.27	0.26	6.4	6.5	2.7	3.0
T_5	45.4	44.8	0.10	0.10	0.25	0.23	6.0	6.1	2.5	2.8
T_6	32.8	36.2	0.12	0.11	0.27	0.28	6.3	6.6	2.8	3.1
T_7	30.0	38.4	0.11	0.13	0.26	0.29	6.5	6.6	2.9	3.0
T_8	45.5	45.7	0.10	0.10	0.20	0.18	6.6	6.9	2.9	2.9
T_9	44.5	45.4	0.09	0.08	0.13	0.10	6.6	6.5	2.8	3.0
T_{10}	45.1	45.8	0.11	0.12	0.26	0.26	5.7	5.6	2.3	2.5
T_{11}	40.8	45.7	0.11	0.11	0.28	0.27	5.3	4.4	2.3	2.1
T_{12}	10.9	10.5	0.04	0.03	0.12	0.11	1.98	1.72	1.12	1.1
F-test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm (±)	1.67	1.92	0.004	0.009	0.01	0.009	0.14	0.19	0.1	0.1
CV (%)	4.89	5.64	0.012	0.027	0.028	0.027	0.41	0.56	0.28	0.29

The treatment details for *Kharif* and *Boro* rice are mentioned in Table 3. CV (%) = coefficient of variation; NS = not significant.

Table 9. Effect of nutrient management on the nutrient uptake (kg ha^{-1}) of *Boro* rice.

Treatments	N Uptake in Grain		N Uptake in Straw		P Uptake in Grain		P Uptake in Straw		K Uptake in Grain	
	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016
T_1	98.9	107.7	57.5	56.4	34.3	38.3 a	28.9 a	30.2 a	28.5	33.0 a
T_2	76.7	81.1	41.2	51.5	28.5	30.8 f	25.3 de	25.8 ef	27.0	30.5 f
T_3	17.3	15.9	18.3	14.2	17.9	17.3 h	15.0 h	17.2 h	15.0	16.3 h
T_4	87.6	100.5	52.4	54.1	25.5	27.8 g	20.8 g	24.3 g	27.4	30.8 d
T_5	86.6	99.9	57.2	56.2	15.3	12.8 i	10.2 i	7.0 i	27.7	30.7 de
T_6	90.1	99.3	55.4	54.4	31.0	33.6 e	24.8 ef	25.3 f	19.9	25.1 g
T_7	87.7	97.2	53.2	52.2	30.6	33.7 d	25.8 cd	26.9 cd	12.1	10.0 i
T_8	88.1	102.4	52.3	54.5	32.1	33.7 d	26.1 c	28.0 b	27.0	31.0 c
T_9	88.7	98.6	52.1	53.6	32.0	34.0 b	24.7 ef	26.4 de	26.9	30.6 ef
T_{10}	87.3	100.3	52.4	55.3	32.0	33.8 c	27.5 b	27.7 bc	26.6	30.5 f
T_{11}	88.7	95.5	51.4	51.1	31.1	33.8 c	24.2 f	26.6 de	27.9	31.9 b
T_{12}	6.3	12.4	16.9	11	4.86	4.16 j	4.29 j	3.45 j	2.92	2.62 j
F-test	NS	NS	NS	NS	NS	**	**	**	**	**
SEm (±)	3.5	3.6	1.7	1.9	1.92	1.67	1.67	2.46	0.67	0.83
CV (%)	11.7	10.2	4.9	5.7	5.64	4.89	4.89	7.22	1.96	2.44
Treatments	K uptake in straw		Zn uptake in grain		Zn uptake in straw		S uptake in grain		S uptake in straw	
	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016	2014–2015	2015–2016
T1	53.3 a	64.3 a	0.19	0.19	0.35 a	0.34 ab	9.2 a	9.3 a	6.4 a	6.7 a
T_1	48.5 d	59.5 c	0.18	0.18	0.32 a	0.33 ab	8.6 ab	8.6 ab	6.4 a	6.3 abc
T_2	37.7 f	36.2 h	0.12	0.14	0.20 bc	0.21 bc	4.7 d	4.7 d	4.2 d	4.5 d
T_3	51.6 b	63.4 b	0.18	0.18	0.31 a	0.34 ab	8.6 ab	8.6 ab	6.2 ab	6.5 ab
T_4	48.7 cd	56.9 e	0.16	0.16	0.27 ab	0.32 ab	8.2 b	8.5 ab	5.6 bc	5.7 c
T_5	37.0 f	41.2 g	0.18	0.18	0.31 a	0.32 ab	8.6 ab	8.7 ab	6.0 ab	6.2 abc
T_6	34.9 g	51.0 f	0.18	0.18	0.33 a	0.35 a	8.9 ab	8.8 ab	6.0 ab	6.3 abc
T_7	48.6 d	63.8 ab	0.13	0.12	0.22 b	0.23 abc	8.8 ab	8.8 ab	6.5 a	6.3 abc
T_8	45.5 e	58.6 d	0.10	0.09	0.11 d	0.10 c	8.3 b	8.4 b	5.0 c	5.9 bc
T_9	50.7 b	56.8 e	0.18	0.18	0.34 a	0.34 ab	6.8 c	6.9 c	3.1 e	3.5 e
T_{10}	49.6 c	56.8 e	0.18	0.18	0.34 a	0.34 ab	4.9 d	4.6 d	2.0 f	2.1 f
T_{11}	12.6 h	11.98 i	0.03	0.02	0.12 cd	0.11 c	1.62 e	1.51 e	1.23 g	1.08 g
F-test	**	**	NS	NS	**	**	**	**	**	**
SEm (±)	1.8	2.67	0.006	0.007	0.012	0.01	0.23	0.25	0.15	0.17
CV (%)	5.28	7.82	0.018	0.021	0.037	0.03	0.69	0.75	0.43	0.49

The treatment details for *Kharif* and *Boro* rice are mentioned in Table 3. CV (%) = coefficient of variation; ** and * significant at $p \leq 0.01$ and $p \leq 0.05$, respectively; NS = not significant; different letters within the continuous columns indicate significant differences at the 1% level of probability.

Table 10. Effect of nutrient management on the nutrient balance of soil after the second year of *Boro* rice.

Treatment	Initial Soil Status (kg/mg ha^{-1})					The 2nd Year of *Boro* Rice (kg/mg ha^{-1})					Nutrient Balance (kg/mg ha^{-1})				
	N	P	K	Zn	S	N	P	K	Zn	S	N	P	K	Zn	S
T_1	230.5	11.2	125.2	0.2	10.5	290.5	31.7	211.2	0.6	27.2	60.0	20.5	86.0	0.4	16.7
T_2	230.5	11.2	125.2	0.2	10.5	242.3	27.9	209.2	0.5	24.9	11.8	16.7	84.0	0.3	14.4
T_3	230.5	11.2	125.2	0.2	10.5	216.4	28.5	208.3	0.4	24.5	−14.1	17.3	83.1	0.2	14.0
T_4	230.5	11.2	125.2	0.2	10.5	286.3	26.6	210.2	0.5	25.5	55.8	15.4	85.0	0.3	15.0
T_5	230.5	11.2	125.2	0.2	10.5	286.6	9.0	207.8	0.5	24.4	56.1	−2.2	82.6	0.2	13.9
T_6	230.5	11.2	125.2	0.2	10.5	285.8	30.3	196.0	0.6	24.4	55.3	19.1	70.8	0.3	13.9
T_7	230.5	11.2	125.2	0.2	10.5	282.8	28.6	112.1	0.6	24.1	52.3	17.4	−13.1	0.3	13.6
T_8	230.5	11.2	125.2	0.2	10.5	282.7	28.3	207.8	0.4	24.2	52.2	17.1	82.6	0.2	13.7
T_9	230.5	11.2	125.2	0.2	10.5	280.7	28.7	204.9	0.1	25.0	50.2	17.5	79.7	−0.1	14.5
T_{10}	230.5	11.2	125.2	0.2	10.5	284.2	29.0	202.4	0.5	16.2	53.7	17.8	77.2	0.3	5.7
T_{11}	230.5	11.2	125.2	0.2	10.5	276.0	27.2	201.9	0.4	3.4	45.5	16.0	76.7	0.2	−7.1
T_{12}	230.5	11.2	125.2	0.2	10.5	214.3	8.8	112.4	0.1	3.2	−16.2	−2.4	−12.8	−0.1	−7.3
STDEV	0.00	0.00	0.00	0.00	0.00	27.99	7.81	36.73	0.17	8.49	27.99	7.81	36.7	0.16	8.49
±SEm	0.00	0.00	0.00	0.00	0.00	8.08	2.26	10.60	0.05	2.45	8.08	2.26	10.6	0.05	2.45

The treatment details for the *Kharif* and *Boro* rice are mentioned in Table 3.

3.3.1. Nitrogen Uptake

In 2014, the highest nitrogen uptake in *Kharif* season rice grain (68.5 kg ha^{-1}) was with the treatment T_1, which was significantly higher than T_2, T_3, T_4, T_5, T_8, and the control (T_{12}); however, T_1 remained statistically on par with T_6, T_7, T_9, T_{10}, and T_{11} (Table 8). In 2015, T_1 also resulted in the maximum N uptake by grain and, it being statistically on par with T_4, T_6, T_8, T_{10}, and T_{11}, was significantly superior to T_2, T_3, T_5, T_7, T_9, and T_{12} (control). Similarly, the nitrogen uptake by straw was also maximum with an ample dose of fertilizer application. In the case of nitrogen uptake by rice straw, T_1 removed the maximum nitrogen and it remained statistically on par with T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, and T_{11} in 2014 and with T_5, T_7, T_8, T_9, T_{10}, and T_{11} in 2015. Interestingly, T_1 remained on par with those treatments that received 80 kg N ha^{-1} in the *Kharif* season. The least quantity of N was removed by the rice straw with the unfertilized control (T_{12}) during both years of experimentation. Greater values of N uptake by the grain and straw were noted with the recommended dose of N fertilizer application for *Kharif* rice (80 kg ha^{-1}) during both the years, and it was probably the proper utilization of applied N fertilizer by crops into biomass (grain and straw yields) production.

During the *Boro* seasons of 2014–2015 and 2015–2016, the highest nitrogen uptake by rice grain (98.8 and 107.6 kg ha^{-1}) was recorded with the treatment T_1 and the least quantity was noted with the unfertilized control (T_{12}) (Table 9). The result of N uptake by grain in 2014–2015 revealed that 100% RDF (T_1) removed the maximum nitrogen from the soil and it was significantly more than T_2, T_3, T_5, and T_{12} (control). The remaining treatments were statistically on par with T_1 in the expression of nutrient uptake by grains of *Boro* rice in 2014–2015. However, in the case of 2015–2016, T_1 registered more nitrogen uptake by grains of *Boro* rice, which was further significantly more than T_2, T_3, T_7, T_{11}, and T_{12} (control). N uptake by *Boro* rice grains was drastically reduced in T_3, which was statistically on par with the unfertilized control (T_{12}) during both years of experimentation. Rice is basically a nutrient-draining crop and in the rice-rice cropping system, the second crop (*Boro* rice) did not get any nitrogen in T_3, probably due to insufficient supply of the primary nutrient (N); thus, the treatment performed poorly.

In 2014–2015, *Boro* rice straw registered its maximum uptake of nitrogen at T_1 and it was statistically on par with T_5, T_6, and T_7. T_{12} (unfertilized control) expressed the least value and it was statistically on par with T_3. Both T_3 and T_{12} (control) were significantly inferior to other treatments in 2014–2015 in N uptake by straw. However, in 2015–2016, T_1 was statistically on par with all other treatments except T_3 and T_{12} (control). The variation in N uptake during two consecutive years among treatments was probably due to variation in yields. In both years, T_3 and T_{12} (control) performed poor in nitrogen uptake by straw because nitrogenous fertilizer was not applied in these treatments. Earlier researchers evidenced that an ample dose of fertilizer application recorded more uptake of nitrogen by grains and straws of rice [40,41].

3.3.2. Phosphorus Uptake

In 2014, the uptake pattern of P was influenced by the yield of *Kharif* rice grain and straw (Table 9). The highest P uptake in rice grain (19.30 kg ha^{-1}) in 2014 was noted with the treatment T_1, which was statistically on par with T_6, T_7, T_8, T_9, T_{10}, and T_{11}. The grains of *Kharif* rice in 2015 also removed the maximum P by T_1; however, it was statistically on par with only T_6 and T_{10}. The lowest quantity of P uptake by the *Kharif* rice grains was recorded with T_{12} (control) during both years. A similar trend in P uptake by rice straw during the *Kharif* season was noted in 2014 and 2015 where the treatment T_1 resulted in their maximum values (11.8 and 14.1 kg ha^{-1}, respectively). In 2014, T_1 registered significantly more nutrient uptake by rice straw during the *Kharif* season than T_3, T_4, T_5, and the control (T_{12}); but T_1 was also statistically on par with T_2, T_6, T_7, T_8, T_9, T_{10}, and T_{11}. The treatment ($N_{80}P_{20}K_{40}Zn_{25}S_{20}$) also removed the maximum P by straw in *Kharif* of 2015, and the treatment is statistically on par with T_2, T_7, T_8, T_9, T_{10}, and T_{11}, but significantly superior to T_3, T_4, T_5, T_6, and the control.

In the case of *Boro* rice, a similar trend was observed in terms of nutrient uptake by rice grain and straw during both the years (2014–2015 and 2015–2016) (Table 10). An ample dose of recommended fertilizer application (T_1) registered significantly more P uptake during both the years by *Boro* rice grains over T_2, T_3, T_4, T_5, and T_{12} (unfertilized control) and the treatment with the application of T_1 remained statistically on par with T_6, T_7, T_8, T_9, T_{10}, and T_{11}. Similarly, straw of *Boro* rice showed maximum P uptake by T_1 and the treatment being statistically on par with T_6, T_7, T_8, T_9, T_{10}, and T_{11} resulted in significantly greater P uptake by the straw of *Boro* rice than T_3, T_4, T_5, and T_{12} (control). Research evidence proved that an ample dose of the recommended dose of nutrient application showed greater uptake of P by grains of rice [42,43].

3.3.3. Potassium Uptake

Potassium uptake by grains of *Kharif* rice was maximum at an ample dose of recommended fertilizer application (T_1) during both years of experimentation (Table 9). The treatment T_1, being statistically on par with T_2, T_4, T_5, T_8, T_9, T_{10}, and T_{11}, registered its significant superiority to T_3, T_6, T_7, and unfertilized control (T_{12}) in the *Kharif* season of 2014 for potassium removal by grains. However, in 2015, only a few treatments, namely T_2, T_9, T_{10}, and T_{11}, remained statistically on par with T_1 in increasing the K uptake by *Kharif* rice grains and the treatment T_1 recorded significantly more uptake of said primary nutrients over the remaining treatments. Further, it was also noted that the treatment T_{12} recorded the least quantity of K during both the years and, in *Kharif* 2015, the rice grains registered a comparatively less amount of K removal because of the non-application of nutrients in the two consecutive years. A similar trend was also noted in K uptake by the straw of *Kharif* rice during both the years as the treatment T_1 showed the maximum uptake. In both the years, straw of *Kharif* rice removed the maximum amount of K with the treatment T_1 and it was statistically on par with T_2, T_4, T_5, T_8, T_9, and T_{10}. In 2014, T_1 remained significantly superior to T_{11}, but in 2015 both the treatments were statistically on par in the removal of K by *Kharif* rice straw.

The data on potassium uptake (kg ha^{-1}) showed that during the *Boro* season (Table 10), the uptake of K in grain was the highest with T_1 and it was statistically on par with T_2, T_4, T_5, T_8, T_9, T_{10}, and T_{11} in 2014–2015. The lowest value was recorded in T_{12} (control) and it was significantly inferior to all other treatments. Interestingly, in 2015–2016, K uptake by the rice grains was less than the previous year with the same treatment (T_{12}) and that clearly indicated the continuous removal of stored nutrients because in the treatment no nutrients were added in the consecutive two years. In the *Boro* season of 2015–2016, an ample dose of nutrients application (T_1) registered the maximum K uptake by rice grains and the treatment was statistically on par with T_4, T_5, T_8, T_9, and T_{11}. Like other major nutrients, K uptake of rice straw during the *Boro* season of 2014–2015 was noted as at a maximum at T_1 and, it being statistically on par with T_2, T_4, T_5, T_8, T_9, and T_{10}, remained significantly superior to T_3, T_5, T_6, T_9, and the unfertilized control (T_{12}) in 2015–2016. However, in 2015–2016, the treatment T_1 was observed to remove significantly more K by *Boro* rice straw than T_3, T_5, T_6, T_7, T_{10}, T_{11}, and T_{12} (control). The results noted that higher removal of K by rice with the recommended dose of fertilizer application [44,45].

3.3.4. Zinc Uptake

Zn uptake (kg ha^{-1}) by *Kharif* rice grain and straw was influenced by nutrient management treatments during two consecutive years of study (Table 9). In 2014, rice grains registered their maximum Zn uptake by T_1, and it remained statistically on par with T_2, T_4, and T_6 and significantly superior to the rest of the treatments. In 2015, T_1 remained statistically on par with T_2 and T_{10} in the uptake of Zn by rice grains; however, T_1 recorded significantly more Zn uptake over other treatments. Similarly, rice straw also recorded its maximum Zn uptake with T_1 during the *Kharif* seasons of 2014 and 2015. In 2014, T_1 was statistically on par with T_4, T_6, and T_{11}; but, in 2015, treatment T_1, being statistically on par with T_6 and T_7, recorded significantly more Zn uptake by the straw of *Kharif* rice over other treatments. Moreover, the control treatment (T_{12}) recorded the lowest uptake of Zn during both the years by grain and straw of *Kharif* rice because of it being devoid of any fertilizer application.

Boro rice grain and straw also showed a similar trend in Zn uptake as the maximum uptake of the micronutrient was noted with an ample dose of recommended fertilizer application (T_1) in both years (Table 10). An ample dose of the recommended fertilizer (T_1) was statistically on par with T_2, T_6, T_7, T_{10}, and T_{11} in Zn uptake by *Boro* rice grains; however, the treatment was significantly superior to the remaining treatments in 2014–2015 and 2015–2016. Similarly, the *Boro* rice straw removed the maximum Zn with the treatment T_1 and it is statistically on par with T_2, T_7, T_{10}, and T_{11}, registering more Zn uptake than other treatments during both years. As noted in the other treatments, the least quantity of Zn uptake by grain and straw of *Boro* rice was recorded with the unfertilized control treatment (T_{12}). The results are in agreement with the research evidence of Mohapatra [38], Pampolinoa et al. [46], and Chandrapala et al. [47], who earlier noted a higher quantity of Zn removal by rice with an ample dose of Zn-containing fertilizers.

3.3.5. Sulphur Uptake

In the rice–rice cropping system, S uptake was influenced by nutrient management in the *Kharif* season (Table 9). During both the years (2014 and 2015), *Kharif* rice grains registered their maximum S uptake by the treatment T_1 and, it being statistically on par with T_2, T_4, T_6, T_7, T_8, and T_9, recorded more S uptake over the remaining treatments. In the case of rice straw, in 2014, T_1 was statistically on par with T_2, T_7, and T_8; and in 2015, the treatment T_1, being statistically on par with T_2, T_4, T_6, T_7, T_8, and T_9, registered significantly more S uptake than the other treatments during the *Kharif* season. As expected, the control treatment (T_{12}, control) recorded the least S uptake by the *Kharif* rice grain and straw and it was significantly inferior to all other treatments during both the years under study.

A similarity was noted in S uptake by *Boro* rice grain and straw during both years (Table 10). The treatment T_1 recorded the maximum S uptake by *Boro* rice grain and straw and it was statistically on par with T_2, T_4, T_6, T_7, and T_8 during both years. However, T_1 was significantly superior to the other treatments in S removal by the grain and straw of *Boro* rice in 2014–2015 and 2015–2016. The treatment with no fertilizer (T_{12}, control) showed significant inferiority over other treatments for S removal by grain and straw of *Boro* rice during both the years under study. The results clearly indicated that application of S was required in the rice–rice cropping system for proper nutrition of the crops. The results conform with the findings of Porpavai et al. [48] and Singh et al [49].

3.4. Nutrient Balance

The initial nutrient status of the soils before transplanting of *Kharif* rice was analysed and recorded (Table 10). The nutrients were added through chemical fertilizers as per the treatments for rice crops in the rice–rice cropping system. The removal of nutrients by the rice crop was quantified after the harvest of each crop during the *Kharif* and *Boro* season in two consecutive years. The nutrient balance was measured after the final harvest of *Boro* rice in 2015–2016. The rice–rice cropping system removed a considerable amount of nutrients during the two years of study and the ample dose of recommended fertilizer application recorded the maximum quantity of nutrient (N, P, K, Zn, and S were considered in the experiment) removal. As expected, the control treatment (no fertilizer application) yielded less with the least nutrient uptake. After completion of two years of the experiment, it was observed that omission of any nutrient, as well as a control treatment, resulted in a negative nutrient balance, which is synonymous with depletion of soil fertility. The results clearly showed that to achieve crop yields on a sustainable basis one would need to apply the recommended fertilizers, and these recommendations should be made based on crop demand (removal) and the inherent soil nutrient-supplying capability. A similar type of observation was earlier noted by researchers [50,51].

As the rice–rice cropping system is the most prevalent system for irrigated lands of the red and lateritic belt of West Bengal, the nutrient balance must be kept into consideration for agricultural sustainability. Further, being a nutrient draining system, rice–rice systems remove a sizable quantity of nutrients, causing multi-nutrient deficiency problems—a threat to sustainable farm output—which is unlike other rice-based cropping systems, such as rice–legume systems, which has the opportunity to replenish a portion of the nutrients (more specifically N) through biological N fixation and nutrient recycling. In the rice–rice cropping system, the soil remains flooded for a long period and in this condition, loss of N and non-availability of Zn further aggravate the dimension to improper plant nutrition. Under rice–rice cropping systems, exogenous application of nutrients is vital for nutrient supply to crops. The experimental results of the present study also revealed that the application of ample doses of recommended nutrients is essential to maintain a positive nutrient balance.

4. Conclusions

Without proper and balanced nutrient management practices, the rice-rice system can prove to be highly unsustainable and can drain the soil nutrients quickly. Hence, understanding the nutrient requirement, nutrient removal, and nutrient balance of this system is essential. Nitrogen, phosphorus, potassium, zinc, and sulphur are the nutrients of topmost priority, as their deficiency is widespread. These nutrients also play a crucial role in deciding crop performance. In the experiment, a rice–rice cropping system was studied concerning different nutrient management options. The imbalanced or insufficient nutrient application affects crop nutrient removal, thus affecting the growth and development of the plant. In addition to this, inappropriate nutrient supply over a long period reduces soil fertility, especially when a nutrient-exhausting cropping system such as a rice–rice cropping system is practised. The treatment where ample nutrients were provided proved to be most effective in improving the growth parameters, yield-attributing characteristics, and yield of rice in both the *Kharif* and *Boro* seasons. Ample nutrient application also helped to replenish the nutrients removed by the rice–rice cropping system. Imbalanced and insufficient nutrient application may make a nutrient-intensive cropping system, such as a rice–rice cropping system, unsustainable and low yielding. Considering this, an ample dose of nutrients in balanced proportions may be recommended to farmers of eastern India to maintain both productivity and agricultural sustainability and also to avoid long-term nutrient deficiencies in the rice–rice cropping systems of the region. Balanced nutrient management in cropping systems, thereby minimizing environmental pollution, is a cost-effective and environmentally friendly approach to target agricultural sustainability.

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Article

The Effect of Soil-Climate Conditions, Farmyard Manure and Mineral Fertilizers on Potato Yield and Soil Chemical Parameters

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Abstract: If available to farmers, potatoes represent a crop classically fertilized with farmyard manure in the Czech Republic. At the same time, potatoes are a crop sensitive to soil–climate conditions. We evaluated the effect of cattle manure (FYM), manure and mineral nitrogen (FYM + N1, FYM + N2), manure and mineral fertilizers (FYM + N1PK, FYM + N2PK, FYM + N3PK) application and the effect of three soil-climatic conditions (Caslav—maize production area with degraded Chernozem, Ivanovice—maize production area with Chernozem, Lukavec—potatoes production area with Cambisol) over four years (2016–2019) on potatoes yield and soil chemical properties. Of all the factors, yields were most affected by location. Lukavec provided the highest average yields (37.2 t ha^{-1}), followed by Ivanovice (23.5 t ha^{-1}) and Caslav (15.5 t ha^{-1}). The second most important factor was the climatic conditions of the year. Fertilization was the third most important parameter. FYM significantly increased yields compared to Control, but applied alone cannot cover the needs of potatoes. Similarly, the application of FYM and N increases yields, but for the highest yields, it is best to apply FYM + NPK (80 kg ha^{-1} N). Co-application of FYM and mineral N fertilizers mitigates the negative impact of mineral N on soil pH.

Keywords: *Solanum tuberosum* L.; cattle manure; mineral N; P and K; weather conditions; soil pH; soil nutrient content; PCA; FA

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1. Introduction

Fertilizer application is the cornerstone of crop production. The origins of fertilization are linked to the Neolithic Revolution when people switched from hunting and gathering to agriculture. People began to settle at the expense of migration, built their first settlements and started to collect various forms of waste in pits located near their houses. Such pits are documented in Sumerian cities, in the period around 6000 BC [1]. More recent research has shown that even in earlier times people used manure and water management to increase crop yields [2]. Even today, organic manures are an essential element of crop production, together with organic and mineral fertilizers. All three groups of fertilizers (organic manures, organic and mineral fertilizers) are characterized by different mechanisms of action on soil and crops. Organic manures have a beneficial effect on the physical, chemical and biological parameters of the soil [3–8], but their nutrient content is relatively low and must therefore be applied in large doses. The composition of individual organic manures is not homogeneous, varying both within and between types (slurries, manures), depending on their origin [9]. The rate of mineralization of manure strongly depends on the type of manure and the climatic conditions. While organic manures with a low C:N ratio (slurries) provide the most nutrients in the first year of application, manures with a high C:N ratio (farmyard manures—FYM) release nutrients to a lesser extent but over a longer period [10]. However, even organic manures can harm the environment, either through over-fertilization or through the presence of undesirable substances that enter the soil and water through the application, such as veterinary pharmaceuticals [11]. Mineral fertilizers have a homogeneous and precisely known composition and their effect is rapid. Mineral fertilizers are behind the success of conventional agriculture and ensure the production of basic raw materials for a wide range of industries. On the other hand, mineral fertilizers represent the source of environmental pollution [12–15], which is one of the aspects negatively influencing the public's view on conventional agriculture and is behind the growing interest in organic farming [16]. Agriculture in the Czech Republic is characterized by an imbalance between livestock and crop production due to the changes in the crop rotations (reduction of perennial fodder crops and cereals in favor of winter rapeseed), reduced animal husbandry (there are areas completely devoid of livestock production), which leads to the low organic manure inputs, and finally by the imbalance between applied mineral nutrients [17] (high application of nitrogen fertilizers, low application of phosphate and potassium fertilizers, Figure 1, and low level of liming [18]). A similar trend for mineral fertilizers can be discerned in neighboring Poland [19]. All these aspects lead to soil degradation, which has been evident in the Czech Republic for a long time [20].

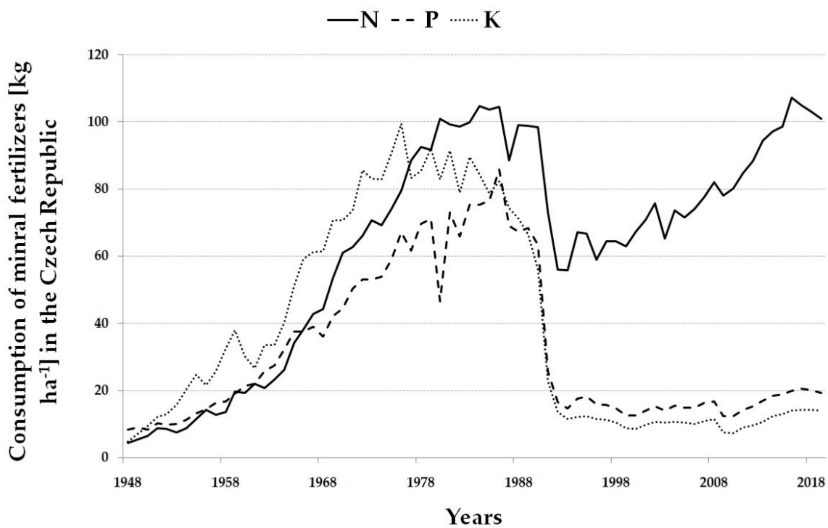

Figure 1. Average consumption of mineral N, P and K (kg ha^{-1}) in the Czech Republic (1948–2019).

While the application of mineral nitrogen and potassium can satisfy crop requirements, it is also associated with negative aspects such as pH reduction and depletion of other nutrients [21–23]. And as the soil pH decreases, the mobility of risk elements (heavy metals, such as Cd, Cu, Mn, Pb and Zn) in the soil increases [15], which can negatively affect the quality of crop production [24].

The yield and quality of crops are not only affected by fertilization and management practices. Soil and climatic conditions also play an important role. Potatoes are one of the most nutrients and moisture demanding crops, requiring high amounts of N due to the poor N efficiency [25]. They tend to prefer lighter soil types, which are found at higher altitudes, receiving higher rainfall, which compensates for the negative aspect of light soils—drying out. Comparing the effect of climatic conditions, both temperature and precipitation play an important role in yield forming. This is especially important nowadays when we are exposed to changing climatic conditions and more frequent occurrences of unusual (extreme or extraordinary) phenomena [26,27]. High temperatures can negatively affect the efficiency of photosynthesis, water management and respiration. However, the temperature seems to play a less important role as a factor affecting potatoes yields than precipitation, which is a more important yield formatting—factor and can compensate for the negative effects of high temperatures [28] and increase fertilizer utilization [29]. The precipitation and soil type are so important factors for potatoes that even the most naturally fertile soils

(Chernozems) in the Czech Republic [30] cannot provide high potato yields without the proper climate conditions. In other words, it means that naturally created barriers strongly limit the farmer's options, regardless of fertilization or farming practices.

In the Czech Republic, potatoes are traditionally fertilized with FYM (if available) in the first line. Manure not only gradually adds nutrients, but in heavy (clay) soils, which are not conducive to good potato growth, it acts as an aerating agent and alleviates soil's heaviness. On the other hand, in light (sandy) soils, manure provides organic matter and nutrients that would otherwise be lacking. Application of manure significantly increases the potatoes yield [31] and also affects yields and soil chemical composition a long time after the manure application [32]. However, organic manures (such as FYM) cannot supply enough nutrients to meet the needs and potential of modern potato varieties. For this reason, it is advisable to apply mineral fertilizers [25,33] or combine organic manures with mineral fertilizers [34]. However, fertilization recommendations cannot be generalized, as each recommendation should be site-specific, based on the soil and climate conditions of the site [35].

Our main research goal was to assess characteristics of the interactions between differentiated fertilization management (seven fertilization treatments) and environmental factors in aspects of its influence on the potato yields and selected soil parameters (pH, N, P, K and soil carbon content—Cox). The fertilization treatments represent different management practices and include 1) unfertilized Control, 2) application of cow manure (FYM), 3, 4) combination of manure and two different mineral nitrogen rates (FYM + N1, FYM + N2), which represents the direction of fertilization without the application of mineral P and K fertilizers, and 5, 6 and 7) the combination of FYM and mineral NPK fertilizers (FYM + N1PK, FYM + N2PK, FYM + N3PK), which represents the combination of manure and all three major mineral fertilizers (against FYM + N treatments). The experiment was conducted between the years 2016 and 2019 (four years) on three sites with different soil and climatic conditions (Caslav—degraded Chernozem, Ivanovice—Chernozem, Lukavec—Cambisol).

2. Results

2.1. Weather Conditions

2.1.1. Caslav

In Caslav, the weather conditions were the main factor influencing yields (see Section 2.2.1, 67% according to the MANOVA). The lowest average yields were recorded in 2018 (7.7 t ha^{-1}, Table 1), which was the season characterised as a season with precipitation very below normal (Table S1). The sum of precipitation was very below normal during April and May and extraordinary below normal during July (Table S1). The year 2018 was also the hottest one. April and August were especially hot, characterized as extraordinary above normal (Table S2), and the whole season was very above normal. This means that 2018 was a very dry and warm year in Caslav, which affected the yield.

2.1.2. Ivanovice

A similar situation was recorded in Ivanovice. Yields here were largely influenced by weather conditions (see Section 2.2.2, 87% according to the MANOVA). The lowest average yields were recorded in 2018 (12.0 t ha^{-1}, Table 1). The 2018 season was characterized as the season with the lowest sum of precipitation (228.5 mm during the season, Table S1). The 2018 season was also the hottest one. With the average temperature of 18.8°C, the 2018 season was extraordinary above the normal season, with two months (April and August) being extraordinary above normal (Table S2). As in Caslav, the combination of unprecedented conditions in 2018 resulted in extraordinary low yields in 2018.

2.1.3. Lukavec

In Lukavec, the lowest average yields were recorded in 2019 (26.3 t ha^{-1}) and 2018 (30.2 t ha^{-1}, Table 1). In both seasons we recorded extraordinary above normal temperatures (April and August in 2018 and June in 2019, Table S2). Also, April (the month of planting) was dry in both years, in 2019 followed by very cold May and extraordinary hot June (Table S1), which negatively affected plant development, resulting in the lowest yields in this season.

Table 1. The effect of the fertilizer treatments on potato yields as affected by the year (2016–2019) and locality (Caslav, Ivanovice, Lukavec).

	Control	FYM	FYM + N1	FYM + N2	FYM + NPK1	FYM + NPK2	FYM + NPK3	Mean
Caslav								
2016	8.6 ± 0.4 a	14.9 ± 1.1 b	20.0 ± 0.9 c	19.9 ± 0.2 c	25.4 ± 1.2 d	32.7 ± 1.2 e	30.0 ± 0.8 e	21.7 ± 1.5 D
2017	7.1 ± 0.7 a	9.3 ± 0.7 ab	10.3 ± 0.3 ab	12.3 ± 0.6 bc	16.2 ± 1.1 cd	19.9 ± 1.0 d	25.3 ± 2.0 e	14.3 ± 1.2 B
2018	4.4 ± 0.2 a	6.6 ± 0.3 a	7.5 ± 0.2 ab	7.9 ± 0.3 abc	8.6 ± 0.6 abc	9.5 ± 0.7 c	9.3 ± 0.4 bc	7.7 ± 0.3 A
2019	10.6 ± 0.4 a	11.8 ± 0.3 b	16.7 ± 0.5 c	20.8 ± 0.7 d	21.3 ± 0.4 de	23.2 ± 0.8 de	23.6 ± 0.6 e	18.3 ± 1.0 C
Mean	7.7 ± 0.6 a	10.6 ± 0.9 b	13.6 ± 1.3 c	15.2 ± 1.4 c	17.9 ± 1.7 d	21.3 ± 2.2 e	22.1 ± 2.1 e	
Ivanovice								
2016	18.6 ± 0.2 a	28.7 ± 0.9 b	30.5 ± 0.8 bc	31.9 ± 0.8 bc	30.9 ± 1.2 bc	34.0 ± 2.2 bc	35.1 ± 2.0 c	29.9 ± 1.1 C
2017	15.1 ± 2.1 a	20.3 ± 1.7 ab	20.7 ± 0.5 ab	23.4 ± 0.9 b	24.4 ± 0.6 b	25.1 ± 1.6 b	23.8 ± 1.3 b	21.8 ± 0.8 B
2018	8.9 ± 1.4 a	11.9 ± 2.6 a	11.0 ± 2.0 a	11.6 ± 2.9 a	12.5 ± 1.9 a	14.2 ± 2.8 a	13.7 ± 0.8 a	12.0 ± 0.8 A
2019	17.2 ± 0.6 a	26.8 ± 1.6 b	32.1 ± 2.2 bc	28.8 ± 1.2 b	33.9 ± 2.5 bc	34.9 ± 2.5 bc	38.3 ± 1.1 c	30.3 ± 1.4 C
Mean	15.0 ± 1.1 a	21.9 ± 1.9 b	23.6 ± 2.3 bc	23.9 ± 2.1 bc	25.4 ± 2.3 bcd	27.1 ± 2.4 cd	27.7 ± 2.6 d	
Lukavec								
2016	20.4 ± 1.8 a	29.1 ± 2.2 a	41.9 ± 2.3 b	48.9 ± 3.7 bc	43.7 ± 2.6 b	49.4 ± 2.9 bc	59.7 ± 1.3 c	41.9 ± 2.5 C
2017	29.6 ± 2.6 a	41.9 ± 1.9 b	45.1 ± 2.0 b	49.6 ± 2.7 bc	59.1 ± 0.9 cd	64.4 ± 0.9 c	64.7 ± 2.8 c	50.6 ± 2.4 D
2018	22.9 ± 1.9 a	29.0 ± 0.4 ab	24.4 ± 0.6 a	26.7 ± 0.7 ab	34.0 ± 1.4 bc	36.6 ± 3.4 c	37.7 ± 0.8 c	30.2 ± 1.2 B
2019	15.1 ± 0.5 a	19.2 ± 0.8 a	25.6 ± 0.5 b	31.3 ± 1.0 c	32.4 ± 1.7 c	30.6 ± 1.5 bc	29.7 ± 1.2 bc	26.3 ± 1.2 A
Mean	22.0 ± 1.6 a	29.8 ± 2.2 b	34.2 ± 2.5 c	39.1 ± 2.9 d	42.3 ± 2.9 de	45.2 ± 3.5 ef	47.9 ± 3.9 f	

The mean values (±standard error) followed by the same letter (a—vertically—comparing the fertilizer treatments, A—horizontally—comparing the years in individual localities) are not statistically different ($p < 0.05$).

Taking a closer look at the effect of precipitation and temperature at each site, the temperature was always strongly and negatively correlated with the yield at all three sites (Caslav–r = −0.76, Ivanovice–r = −0.86, Lukavec–r = −0.62), while precipitation was positively correlated, very weakly at Caslav (r = 0.25), moderately at Ivanovice (r = 0.65) and at Ivanovice (r = 0.75).

2.2. Potato Yields

The potato yields were significantly affected by locality (d.f. = 2, F = 1412, $p < 0.001$, the factor "locality" affected the potato yields by 66%), year (d.f. = 3, F = 359, $p < 0.001$, 17%), fertilizer treatment (d.f. = 6, F = 192, $p < 0.001$, 9%), locality × year interaction (d.f. = 6, F = 162, $p < 0.001$, 7%), followed by the fertilizer treatment × year and locality × fertilizer treatment × year interaction (0.3% together). Thus, the results show that potato yields were most influenced by the location of cultivation, then by the factor year, followed by fertilization. The lowest average yields were harvested in Caslav (15.5 t ha^{-1}), followed by Ivanovice (23.5 t ha^{-1}) and Lukavec (37.2 t ha^{-1}). All three results are statistically significantly different. The lowest average yields were recorded in 2018 (16.6 t ha^{-1}), followed by 2019 (25.0 t ha^{-1}), 2017 (28.9 t ha^{-1}), and 2016 (31.2 t ha^{-1}). All four results are statistically significantly different. Fertilization treatments provided average yields in ascending order: Control (14.9 t ha^{-1}), FYM (20.8 t ha^{-1}), FYM + N1 (20.8 t ha^{-1}), FYM + N2 (26.1 t ha^{-1}), FYM + NPK1 (28.5 t ha^{-1}), FYM + NPK2 (31.2 t ha^{-1}), FYM + NPK3 (32.6 t ha^{-1}). From Control to FYM + NPK1 treatments, all results are statistically significantly different. Yields provided by FYM + NPK2 and FYM + NPK3 are statistically insignificant.

2.2.1. Caslav

In Caslav, the potato yields were significantly affected by the year (d.f. = 3, F = 421, $p < 0.001$, 67%), followed by the fertilizer treatment (d.f. = 6, F = 191, $p < 0.001$, 30%) and year × fertilizer treatment interaction (d.f. = 18, F = 17, $p < 0.001$, 3%). The lowest mean yields were harvested in 2018 (7.7 t ha^{-1}), while the highest harvest was recorded in 2016 (21.7 t ha^{-1}). Each year results are statistically different (Table 1). Comparing the fertilizer treatments, the mean yields ranged from 7.7 (Control) to 22.1 (FYM + NPK3) t ha^{-1} (Table 1). Application of FYM significantly increased the potato yields when compared with the unfertilized Control (+2.9 t ha^{-1}). The application of FYM with mineral N treatments (FYM + N1, FYM + N2) slightly (and significantly) increased the yields when compared with the FYM (Table 1). The addition of PK fertilizers resulted in significantly higher yields when compared with FYM + N treatments. Finally, no difference was found between the FYM + NPK2 and FYM + NPK3 treatments (Table 1), showing that the dose of 80 kg ha^{-1} N applied together with mineral PK fertilizers results in high yields and is optimal (application of 120 kg ha^{-1} N is not necessary, the potato yields are not significantly different).

2.2.2. Ivanovice

In Ivanovice, the potato yields were significantly affected by the year (d.f. = 3, F = 178, p < 0.001, 87%), fertilizer treatment (d.f. = 6, F = 25, p < 0.001, 12%), and the year × fertilizer treatment interaction (d.f. = 18, F = 2, p < 0.001, 2%). The lowest mean yields were harvested, as in Caslav, in 2018 (12.0 t ha^{-1}), due to the poor weather conditions. The highest yields were recorded in 2016 (29.9 t ha^{-1}) and 2019 (30.3 t ha^{-1}, Table 1). If we compare the fertilization treatments, we can see that the trend is very similar to Caslav, i.e., yields increase with increasing nutrient inputs. In the case of Ivanovice, however, the differences between the treatments are not so sharp (are overlapping) and all three FYM + NPK treatments provided comparable results. Moreover, the application of FYM resulted in yields comparable to those of all treatments up to FYM + NPK1 (Table 1). The explanation lies in the soil and climatic conditions. In terms of climate, both sites (Caslav and Ivanovice) are comparable. In terms of soil, in Caslav, the crops are grown on degraded chernozem, which is a soil poorer in nutrients (compared to chernozem in Ivanovice) and the crops respond very well and willingly to the nutrients supplied. In Ivanovice the soil is naturally fertile, it is one of the best soils in the Czech Republic, therefore the response of potatoes to the supplied nutrients (via fertilizers) is not so significant and the application of FYM is sufficient to obtain a satisfactory harvest (the yield difference between FYM and FYM + NPK1 is only 3.5 t ha^{-1} and the difference is insignificant).

2.2.3. Lukavec

In Lukavec, the potato yields were significantly affected by the year (d.f. = 3, F = 236, p < 0.001, 70%), fertilizer treatment (d.f. = 6, F = 91, p < 0.001, 27%) and year × fertilizer treatment interaction (d.f. = 18, F = 8, p < 0.001, 3%). The lowest potato yields were recorded in 2019 (26.3 t ha^{-1}), while the highest average yields were harvested in 2017 (50.6 t ha^{-1}, Table 1). As in the previous two sites, average yields increased with the dosage of nutrients supplied (Table 1). Unfertilized Control provided the lowest average yields (22.0 t ha^{-1}), while the highest yields were harvested when FYM + NPK3 was applied (47.9 t ha^{-1}). The FYM + NPK2 treatment (45.2 t ha^{-1}) provided comparable potato yields to the FYM + NPK3 treatment, the difference between the two treatments was 2.7 t ha^{-1} and was not statistically significant (Table 1). The application of 80 kg of mineral N, together with PK fertilizers (FYM + NPK2) is sufficient to achieve decent yields and a 50% increase in the dose of mineral N (FYM + NPK2—FYM NPK3) is not associated with a significant increase in yields.

2.3. Soil Properties

2.3.1. Caslav

The average P concentration in Caslav ranged from 46 mg kg^{-1} (FYM) to 158 mg kg^{-1} (FYM + NPK2), Table 2. No statistically significant differences were observed for the treatments without mineral P (Control, FYM, FYM + N2) (Table 2). Thus, without mineral P application, soil P concentrations were low (Control, FYM) or suitable (FYM + N2). It must be said that the average concentration of 55 mg kg^{-1} (FYM + N2) is at the very lower end of the range for classification as "Suitable". Thus, without mineral phosphate fertilizer application, the P concentration in the soil is poor and significantly affects potato yields (statistically significant difference between FYM + N and FYM + NPK treatments, Table 1).

Table 2. The long–term effect of the fertilization treatments on soil P, K, Mg and Ca (mg kg^{-1}) concentrations.

	P	P Assess.	K	K Assess.	Mg	Mg Assess.	Ca
Caslav							
Control	49 ± 12 ^{A}	Low	120 ± 14 ^{A}	Suitable	145 ± 26 ^{A}	Suitable	2888 ± 118 ^{A}
FYM	46 ± 6 ^{A}	Low	135 ± 16 ^{A}	Suitable	136 ± 4 ^{A}	Suitable	3777 ± 570 ^{A}
FYM + N2	55 ± 11 ^{A}	Suitable	141 ± 11 ^{A}	Suitable	156 ± 6 ^{A}	Suitable	2802 ± 94 ^{A}
FYM + NPK2	158 ± 9 ^{B}	High	221 ± 21 ^{B}	Good	164 ± 3 ^{A}	Good	2950 ± 198 ^{A}
Ivanovice							
Control	66 ± 10 ^{A}	Suitable	181 ± 6 ^{A}	Good	204 ± 17 ^{A}	Good	4102 ± 152 ^{A}
FYM	169 ± 15 ^{C}	High	370 ± 27 ^{C}	High	234 ± 10 ^{A}	Good	4131 ± 166 ^{A}
FYM + N2	117 ± 12 ^{B}	High	288 ± 21 ^{B}	Good	252 ± 14 ^{A}	Good	4232 ± 152 ^{A}
FYM + NPK2	226 ± 8 ^{D}	Very high	447 ± 17 ^{C}	Very high	236 ± 5 ^{A}	Good	4150 ± 226 ^{A}
Lukavec							
Control	44 ± 1 ^{A}	Low	107 ± 6 ^{A}	Suitable	109 ± 9 ^{A}	Suitable	2050 ± 82 ^{A}
FYM	90 ± 8 ^{B}	Good	147 ± 8 ^{BC}	Suitable	126 ± 7 ^{A}	Suitable	2125 ± 96 ^{A}
FYM + N2	46 ± 3 ^{A}	Low	123 ± 5 ^{AB}	Suitable	113 ± 9 ^{A}	Suitable	2214 ± 124 ^{A}
FYM + NPK2	164 ± 5 ^{C}	High	167 ± 4 ^{C}	Suitable	104 ± 9 ^{A}	Low	2182 ± 98 ^{A}

The mean values (±standard error) followed by the same letter (^{A}—vertically—comparing the fertilizer treatments) are not statistically different ($p < 0.05$).

A similar situation occurred in the case of K, with average soil K values ranging from 120 mg kg^{-1} (Control) to 221 mg kg^{-1} (FYM + NPK2). Again, the differences between Control, FYM and FYM + N2 were not significant (Table 2). In contrast, the application of mineral K fertilizers significantly increased soil K concentration to the "Good" level. Also, in the case of yields, we can see here a significant role (together with P) of mineral K, as the differences between treatments with and without mineral P and K fertilizers are significant (Table 1).

In the case of Mg and Ca, fertilizer application did not play a significant role and the differences between the measured concentrations were not statistically significant (Table 1). The average Mg concentration ranged from 136 mg kg^{-1} (FYM) to 164 mg kg^{-1} (FYM + NPK2). Mean Ca concentrations ranged from 2802 mg kg^{-1} (FYM + NPK2) to 3777 mg kg^{-1} (FYM).

Mean soil pH was not significantly different between fertilizer treatments and ranged from 6.51 (FYM + N2) to 6.85 (FYM). Similarly, the concentrations of Cox and Ntot were not significantly different between fertilization treatments and ranged from 1.17% (Control) to 1.29% (FYM + NPK2) for Cox and from 0.15 (Control) to 0.17% (FYM + NPK2) for Ntot (Table 3).

2.3.2. Ivanovice

The P concentration in Ivanovice was significantly dependent on the fertilization treatment, with statistically different values in each fertilization treatment (Table 2). The lowest values were recorded in the unfertilized Control (66 mg kg^{-1}, suitable), followed by FYM + N2 (117 mg kg^{-1}, high), FYM (169 mg kg^{-1}, high) and the highest concentration was in the FYM + NPK2 treatment (226 mg kg^{-1}, very high).

The same situation occurred in the case of K. The lowest concentration was measured in Control (181 mg kg^{-1}), followed by FYM + N2 (288 mg kg^{-1}), FYM (370 mg kg^{-1}) and FYM + NPK2 (447 mg kg^{-1}). Statistically significant differences were recorded between the Control (A), FYM + N2 (B) and FYM (C) treatments together with FYM + NPK2 (C) (Table 2, fertilizer treatments followed by the same letter are statistically insignificant).

As in the previous case, fertilizer application had no significant effect on Mg and Ca concentrations, the differences were not statistically significant. The results of soil analyses for these two elements are shown in Table 2.

Table 3. The long–term effect of fertilization on the value of soil pH, Cox (%) and Ntot (%) content.

	pH (KCl)	C_{ox} (%)	N_{tot}
Caslav			
Control	6.57 ± 0.03 ^{A}	1.17 ± 0.06 ^{A}	0.15 ± 0.01 ^{A}
FYM	6.85 ± 0.14 ^{A}	1.20 ± 0.05 ^{A}	0.15 ± 0.01 ^{A}
FYM + N2	6.51 ± 0.07 ^{A}	1.20 ± 0.05 ^{A}	0.16 ± 0.01 ^{A}
FYM + NPK2	6.53 ± 0.17 ^{A}	1.29 ± 0.09 ^{A}	0.17 ± 0.01 ^{A}
Ivanovice			
Control	6.58 ± 0.14 ^{A}	1.67 ± 0.03 ^{A}	0.20 ± 0.01 ^{A}
FYM	6.69 ± 0.08 ^{A}	1.92 ± 0.06 ^{B}	0.23 ± 0.01 ^{A}
FYM + N2	6.62 ± 0.14 ^{A}	1.95 ± 0.03 ^{B}	0.23 ± 0.01 ^{A}
FYM + NPK2	6.63 ± 0.11 ^{A}	2.07 ± 0.05 ^{B}	0.24 ± 0.01 ^{A}
Lukavec			
Control	5.84 ± 0.06 ^{A}	1.41 ± 0.05 ^{A}	0.19 ± 0.01 ^{A}
FYM	5.88 ± 0.12 ^{A}	1.72 ± 0.06 ^{AB}	0.23 ± 0.01 ^{B}
FYM + N2	5.74 ± 0.08 ^{A}	1.72 ± 0.03 ^{AB}	0.23 ± 0.01 ^{B}
FYM + NPK2	5.83 ± 0.05 ^{A}	1.82 ± 0.13 ^{B}	0.23 ± 0.01 ^{B}

The mean values (±standard error) followed by the same letter (^{A}—vertically—comparing the fertilizer treatments) are not statistically different ($p < 0.05$).

Soil pH was not affected by fertilization and ranged from 6.58 (Control) to 6.69 (FYM), Table 3. The Cox content was significantly affected by fertilization. The lowest concentration was measured in Control (1.67%), which was statistically significantly lower than in the other treatments, which were not significantly different from each other. Cox concentrations in these treatments ranged from 1.92% (FYM) to 2.07% (FYM + NPK2). In the case of Ntot, we did not record statistically significant differences between the treatments and ranged from 0.20% (Control) to 0.24% (FYM + NPK2), Table 3.

2.3.3. Lukavec

In Lukavac, the lowest P concentration was recorded in the Control (44 mg kg^{-1}), which was statistically comparable to the value of 46 mg kg^{-1} (FYM + N2). A higher P concentration was measured in the FYM treatment (90 mg kg^{-1}), which provided a statistically significantly lower yield than FYM + N2 (Table 2), and thus we assume that P originating from FYM was not fully utilized in the case of the FYM treatment, resulting in a higher P concentration in the soil. The highest soil P concentrations were then observed in the FYM + NPK2 treatment, providing the second highest yields (Table 2), and the amount of P supplied was both sufficient to cover the requirements of the potato for high yield formation and sufficient to maintain high soil P levels.

The K concentrations varied significantly between fertilization treatments. The lowest concentration was recorded in Control (107 mg kg^{-1}), followed by FYM + N2 (123 mg kg^{-1}), FYM (147 mg kg^{-1}) and FYM + NPK2 (167 mg kg^{-1}).

In the case of Mg and Ca, the situation in Lukavac was similar to that in Caslav and Ivanovice; fertilizer application did not affect the concentration of these two elements in the soil. The results of soil analyses are shown in Table 2.

Soil pH was not significantly affected by fertilization treatments and ranged from 5.74 (FYM + N2) to 5.88 (FYM), Table 3. In the case of Cox, a significant difference was observed only between Control (1.41%) and FYM + NPK2 (1.82%). In the case of Ntot, the lowest concentration was measured in Control (0.19%), which was statistically significantly lower compared to the other fertilization treatments. For those, the concentration of Ntot was 0.23% (Table 3).

2.4. PCA and FA Results

In the plot of component weights PC1 and PC2 (Figure 2, top left) we can see that the first two axes are significant and together draw 91% of the variability. The PC1 axis in Figure 2, showing the relationship between PC1 and PC2 (Figure 2), characterizes the content of K, Mg and P, which are elements located in the plane with this axis and are strongly correlated with it (K–r = −0.96, Mg–r = −0.86, P–r = −0.81), as well as Cox, a parameter correlated at r = −0.80. Furthermore, there is a significant correlation with Ca on the PC1 axis (r = −0.74). There is a clearly significant correlation with yield (r = −0.95), pH (r = −0.88) and Ntot (r = 0.71) on the PC2 axis. There is no significant correlation on the PC3 axis.

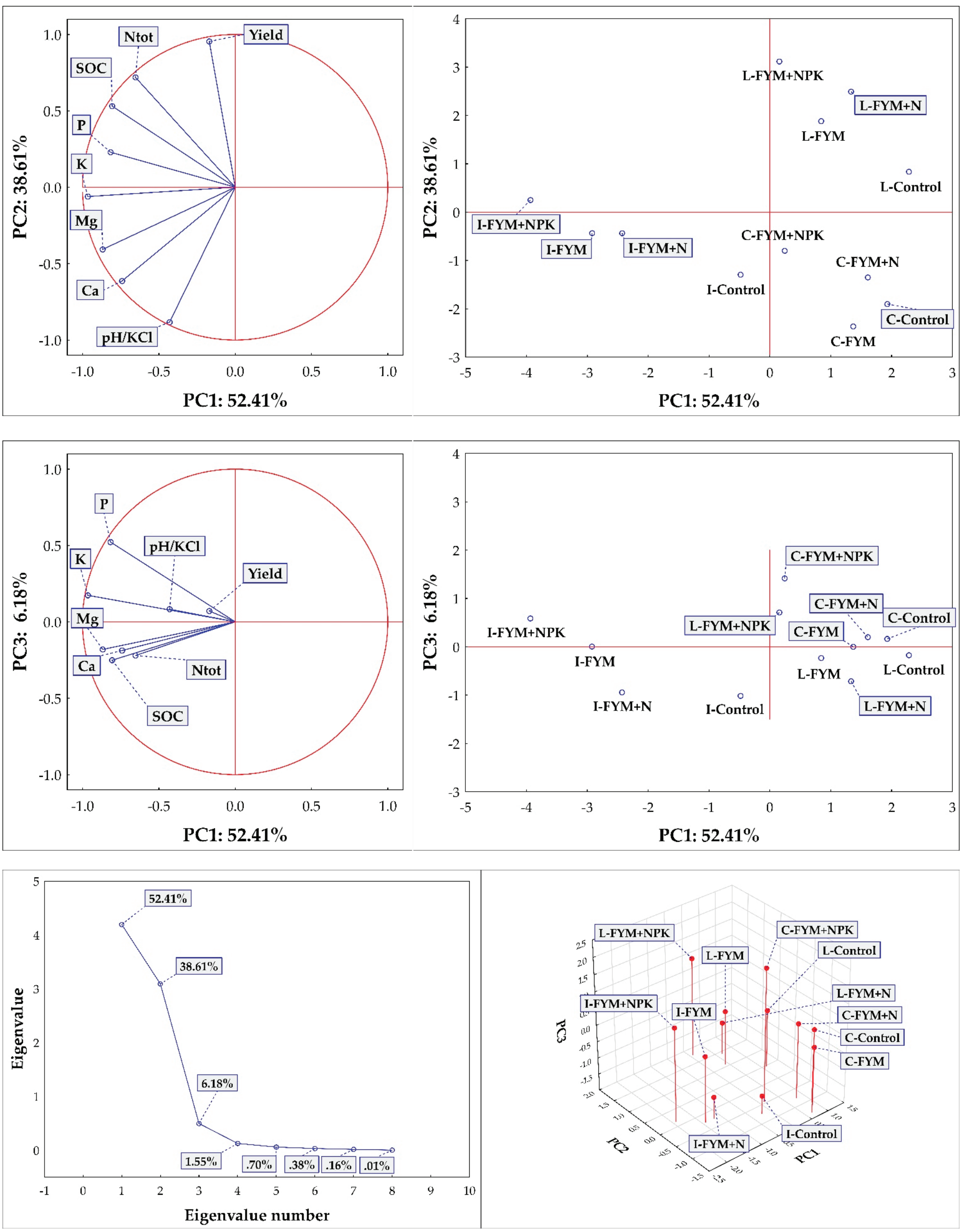

Figure 2. The results of the PCA analyses, including the eigenvalue of the parameters (Figure 2, left bottom side). Note: I—Ivanovice, C—Caslav, L—Lukavec, FYM—farmyard manure, FYM + N represents the FYM + N2 fertilizer treatment,

FYM + NPK represents the FYM + NPK2 treatment, pH—soil reaction; P—phosphorus; K—potassium; Ca—calcium; Mg—magnesium; SOC—soil organic carbon—Cox; Nt—soil nitrogen content (Ntot).

In the component scatter diagram (Figure 2, top right), the sites (Caslav, Lukavec and Ivanovice) and fertilization treatments (C, FYM + N and FYM + NPK) are clearly located along the PC1 axis. The Ivanovice site is significantly to the left along the PC1 axis (highest available nutrient contents, highest Cox, Ntot and pH and lower yields compared to the other two sites—Lukavec and Caslav). The highest contents of available nutrients P and K and Cox and Nt contents, together with yields, were always recorded in the FYM + NPK treatment. And this is on all sites—the FYM + NPK treatment is always significantly more distinct within each cluster—site compared to the Control treatment, where we can find the lowest contents of available nutrients, Cox and Ntot. The FYM and FYM + N treatments at Lukavec and Ivanovice are very similar (clusters of variants close to each other). At the Caslav site, the Control, FYM and FYM+N treatments are very similar (a cluster of treatments close to each other), which is mainly due to the soil type at the site (degraded Chernozem). However, the differentiation within the PC2 axis is also significant. The Lukavec site is significantly different (Figure 1, upper right corner) from the other two sites (Ivanovice and Caslav). This is mainly due to two parameters—significantly lower pH (which suits the potatoes) and significantly higher yields. Lukavec represents a typical potato growing area, while Caslav and Ivanovice are mainly maize growing areas, more suitable for C4 crops.

Factor analysis (FA, Figure 3) confirmed the PCA results and differentiated, similarly to PCA, groups of sites and fertilization treatments. Factor weights explain the correlations between factors and traits (Table 4). They represent the most important information on which the interpretation of the factors is based. It can be said that Factor 1 clearly describes Yield and also soil properties such as Cox, Ntot and additionally P content (significantly higher Cox, Ntot and P content in the FYM + NPK treatment compared to Control at all three sites and also the highest yields at Lukavec compared to Ivanovice and Caslav). Factor 2 clearly describes the content of accessible nutrients (Ca, Mg and K) and the pH value (significant differentiation of sites—the highest pH value, or Ca content, was always recorded in Ivanovice and Caslav, the lowest in Lukavec). Communality represents the proportion of trait variability expressed by the factors in question. The communalities are similar to the R2 value obtained when the original traits are explained by the regression of the selected factors [36]. The contribution of Factor 1 and Factor 2 to communality shows how communality takes high values (more than 0.9). Thus, the trait values are very well accounted for by the proposed factor model (Table 4).

2.5. Linear Regression Model Results

The results of the soil analyses showed a linear regression relationship (data from all three sites) of Ntot content on Cox (Figure 4). The equation of the straight line relating the Ntot and Cox is estimated as: Ntot = (0.0532) + (0.0933) $\times$ Cox (using the 48 observations in the dataset). The statistical characteristics of the linear regression are as follows: r = 0.8675, R^2 = 0.7526, MEP = 0.003, AIC = $-$383.7570. The linear regression model is significant according to Fisher–Snedecor test of model significance (F = 139.9522, quantile F = 4.0517, p = 1-4934E-015). The linear regression model is correct according to Scott's multicollinearity criterion (SC = 0.2811). Residues show homoscedasticity (Cook–Weisberg test of heteroscedasticity). Residues have a normal distribution (according to the Jarque–Berr test of normality). Negative autocorrelation of residues was not demonstrated (according to the Durbin–Watson autocorrelation test). There is no apparent trend in the residuals. The y-intercept, the estimated value of Ntot when Cox is zero, is 0.0532 with a standard error of 0.0129. The slope, the estimated change in Ntot per unit change in Cox, is 0.0933 with a standard error of 0.0079. The estimated slope is 0.0933. The lower limit of the 95% confidence interval for the slope is 0.0774 and the upper limit is 0.1092. The estimated intercept is 0.0532. The lower limit of the 95% confidence interval for the intercept is 0.0273 and the upper limit is 0.0790.

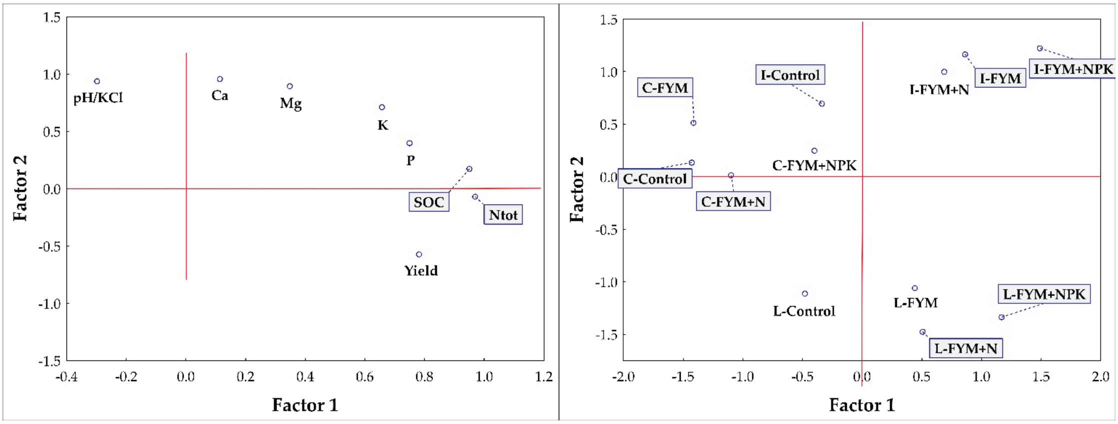

Figure 3. The FA (rotation: varimax normalized) of studied parameters (pH, Cox, Ntot, nutrients and yield) as affected by locality between the years 2016–2019. Note: pH—the value of the soil reaction; P—phosphorus; K—potassium; Ca—calcium; Mg—magnesium; SOC—soil organic carbon—Cox; Nt—soil nitrogen content (Ntot).

Table 4. Factor weights and contributions of given factors to the communality for individual characters after normalized Varimax rotation for production (yield) and soil parameters.

Variable	Factor Weights		Contribution of Factors		
	Factor 1	Factor 2	Factor 1	Factor 2	Communality
pH (KCl)	−0.2978	0.9357	0.0887	0.9644	0.9876
P	0.7492	0.3972	0.5614	0.7192	0.9473
K	0.6569	0.7101	0.4315	0.9359	0.9607
Ca	0.1134	0.9560	0.0128	0.9268	0.9914
Mg	0.3480	0.8936	0.1211	0.9196	0.9397
Cox	0.9498	0.1717	0.9022	0.9317	0.9983
Ntot	0.9693	−0.0704	0.9396	0.9445	0.9977
Yield	0.7811	−0.5738	0.6101	0.9394	0.9336

Furthermore, a statistically significant linear regression relationship (data from all 3 sites) of Yield on soil Ntot content was demonstrated (Figure 5). The equation of the straight-line relating Yield and Ntot is estimated as: Yield = (−13.9802) + (171.7947) × Ntot using the 42 observations in the dataset. The statistical characteristics of the regression are as follows: r = 0.6129, R^2 = 0.3756, MEP = 61.1830, AIC = 173.0356. The model is significant according to the Fisher-Snedecor test of model significance (F = 24.0662, quantile F = 4.0847, $p = 1.5981 \times 10^{-5}$). The model is correct according to Scott's multicollinearity criterion (SC = 0.2645). The residuals show homoscedasticity (Cook-Weisberg test for heteroscedasticity). The residuals have a normal distribution (Jarque-Berr normality test). Residuals are not autocorrelated (Durbin-Watson autocorrelation test). There is no trend in the residuals. The y-intercept, the estimated value of Yield when Ntot is zero, is −13.9802 with a standard error of 7.0537. The slope, the estimated change in Yield per unit change in Ntot, is 171.7947 with a standard error of 35.0191. The estimated slope is 171.7947. The lower limit of the 95% confidence interval for the slope is 101.0184 and the upper limit is 242.5710. The estimated intercept is −13.9802. The lower limit of the 95% confidence interval for the intercept is −28.2362 and the upper limit is 0.2758.

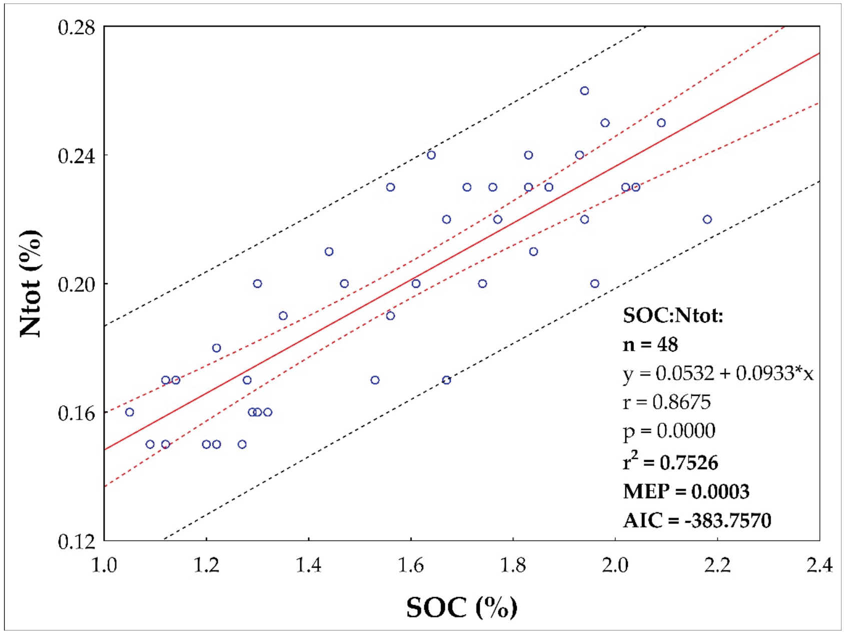

Figure 4. The regression linear relationship between the Cox (SOC) and Ntot between the years 2016 and 2019 (all three study sites—Caslav, Ivanovice, Lukavec).

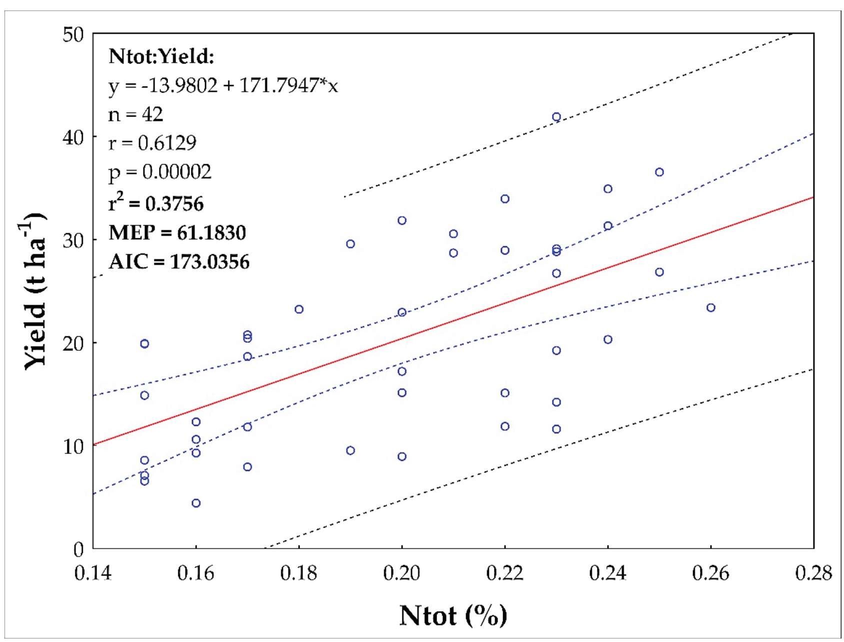

Figure 5. The regression linear relationship between the Ntot and Yield between the years 2016 and 2019 (all three study sites).

3. Discussion

Successful cultivation of quality potatoes is significantly influenced by the location with suitable soil and climatic conditions. The location significantly influences not only the yield itself but also the chemical composition of the potatoes [37–40]. Potatoes thrive on higher sites with higher rainfall and lower temperatures [39], with light soils. The negative impact of unfavourable climatic conditions (lower precipitation and higher temperatures) can be partly offset by the soil and its fertility. This is confirmed by our results, where the lowest average yields were recorded in Caslav (Table 1), a location with similar climatic conditions to Ivanovice, but with soil (degraded Chernozem) poorer in nutrients and low in soil carbon content (Tables 2 and 3). In Ivanovice we can encounter similar climatic conditions as in Caslav, but the soil type here is Chernozem, a much more fertile soil compared to the degraded Chernozem found in Caslav. The difference in fertility (soil properties) between the two soils is due to the conditions of their formation [41]. The soil thus corrects the effect of climatic conditions (see Control results, Table 1), which resulted in higher yields than in Caslav. The highest yields were obtained in Lukavec, which offers the best natural conditions for potato cultivation. This finding is confirmed by PCA analysis (see Figure 2), where the Lukavec locality is significantly different (right upper corner) from the Ivanovice and Caslav localities. This is due to the Lukavec site having statistically significantly higher yields (on the PC2 axis of the component weights plot—significant correlation with Yield, $r = -0.95$), compared to the Caslav and Ivanovice sites. The Lukavec site is a typical potato production area compared to the Caslav and Ivanovice sites (maize production area). Here we can record average yields of 22 t ha^{-1} for the unfertilized Control (Table 1), which is also the average yield of early potatoes in the Czech Republic between the years 2015 and 2019 (the average yield of other potatoes in the Czech Republic was 28 t ha^{-1} between the years 2015 and 2019 [42]).

Weather conditions were the factor that most influenced potato yields at each of the three sites (see MANOVA results, Sections 2.2.1–2.2.3, first two lines), indicating potatoes sensitivity to weather and climate changes [43]. Both precipitation and temperature play an important role during the season and before the season's start [28,43]. According to [44], night temperatures around 17 °C represent the optimum during the tuber formation process, while warmer temperatures significantly decrease the upcoming yields. The weather conditions not only affect the sizes and yields, but also the chemical composition of the potatoes [45]. When potatoes are subjected to stress conditions, whether caused by temperature, precipitation or a combination of these, potatoes respond with lower plant size, lower leaf area and cell membrane stability [46], resulting in lower yields. Such cumulative stress conditions were particularly evident in Caslav and Ivanovice in 2018, a year with exceptionally low yields (Table 1). It was the year characterized by very low precipitation and very high temperatures (Tables S1 and S2). Together with generally less suitable soil and climatic conditions, this occurrence of abnormal weather conditions resulted in average yields of 7.7 t ha^{-1} (Caslav) and 12.0 t ha^{-1} (Ivanovice). In Lukavec, we have recorded particularly low yields in 2018 (30.2 t ha^{-1}) and especially in 2019 (26.3 t ha^{-1}). The dry and warm start of the season in 2018 (Tables S1 and S2) slowed down the initial development of potatoes, but good precipitation in the following two months compensated such situation. In 2019, however, there was a spike in temperatures in May and June, when a very cold May was followed by an extraordinary warm June (Tables S1 and S2). We believe that it was this particular rapid development, coupled with the two extremes, that caused the unusually low yields in 2019, and the relatively normal conditions in the following months of the season did not help to restore the damages.

Fertilization was the second important factor that significantly affected potato yields at all locations (Table 1). Fertilization also affected soil properties (Tables 2 and 3). In recent times, when livestock and crop production in the Czech Republic was in balance, potatoes represented (together with sugar beet) a crop traditionally fertilized with farmyard manure (FYM). Nowadays, this balance is disturbed as many companies do not keep livestock and their crop production strongly depends only on nitrogen from mineral fertilizers [17,47].

The combination of FYM and potatoes was (and still is, if FYM is available) a win-win solution, making potatoes an excellent pre–crop because manure positively modifies soil properties [31,48,49] and slowly releases all macronutrients [10], especially P, K and S [50]. The positive effect of FYM on crop yields and soil properties is well summarized in this meta-analysis [3]. According to [51], the recovery rate of K from FYM by crops vary between 24–26%. In our case, FYM application was always associated with higher yields (compared to unfertilized Control, Table 1). Statistically significant differences were not observed in each year, but over the entire study period, FYM provided significantly higher yields at each site. However, FYM application did not provide enough nutrients (N) to fulfil the yield potential of potatoes, especially in Caslav and Lukavec. The demonstrated dependence (linear regression model) of Ntot content in soil on yield is also related to N application to soil, including N mineralization in soil (Figure 4, r = 0.6129, R^2 = 0.3756). Our obtained Pearson correlations (r) is higher compared to the study of authors [52] who considered r = 0.16 between crop yield in Premslin near Rostock in Germany. The process of mineralization (release of nutrients from FYM into the soil) strongly depends on soil and climatic conditions and can be strongly inhibited in the presence of inconvenient conditions, such as lack of precipitation. Therefore, the addition of mineral N significantly increased potato yields, especially at less fertile sites (Caslav, Lukavec). The FYM + NPK combinations significantly increased yields compared to the FYM + N treatments, again, especially in Caslav and Lukavec. This was confirmed by PCA and FA analysis (see Figures 2 and 3), with the FYM + NPK treatments significantly higher within each subcluster on the PCA axis compared to Control and FYM + N. In Ivanovice, the differences were not so pronounced; the response of the potatoes to the fertilizer supplied was not so strictly noticeable due to the naturally fertile soil. In all cases, the difference between 80 (N2) and 120 (N3) kg N ha^{-1} was not statistically significant and 80 kg N ha^{-1} was sufficient to achieve good (reasonable) yields. The application of mineral P and K fertilizers covered the needs of potatoes to fulfil their potential, especially in less fertile soils (the difference in FYM + N treatments is significant in Caslav and Lukavec, Table 1) and left enough nutrients in the soil for the upcoming crop. This option represents the optimal form of fertilization to achieve high yields and ensure soil fertility. From the soil point of view, it is interesting that we did not observe any difference in pH values (Table 3). In conditions where only N fertilizers are applied to the soil, without the addition of organic matter (or with the addition of small doses of organic matter), the soil becomes acidic [15,21,22,53]. The application of FYM thus reduces the negative effects of mineral N fertilizers on soil pH [8,54]. The addition of FYM and FYM together with mineral fertilizers also increases soil carbon and nitrogen content (Table 3) [55], although the differences were not statistically significant everywhere. This is confirmed by the PCA analysis (Figures 2 and 3), where the highest SOC and Ntot. the content was always recorded in FYM + NPK and FYM treatments, respectively, in all three localities (Caslav, Ivanovice, Lukavec, the FYM + NPK treatments are always significantly separated within each cluster–location, the FYM treatment is always second in order following the FYM + NPK treatment within PC1) compared to the Control treatment (lowest Cox and Ntot contents). Related to this finding is the demonstrated dependence (linear regression model) of content between Cox and Ntot in soil (Figure 3, r = 0.8675, R^2 = 0.7526). From this point of view, the combined application of FYM with mineral NPK represents the optimal form of fertilization to achieve high yields and ensure soil fertility.

4. Materials and Methods

4.1. General Experiment Description

In 1955, three long–term field experiments were established to study the effect of twelve different fertilizer treatments and three soil–climate conditions on yield and quality parameters of arable crops and soil properties. According to Köpper—Geiger climate classification [56], all three sites are located in warm—summer humid continental climate (Dfb). The locations are Caslav (263 m a.s.l., 49°85′ N, 15°40′ E, soil type—calcic degraded Chernozems, arable layer: 40–45 cm), Ivanovice (225 m a.s.l., 49°19′ N, 17°05′ E, soil type—

leptic Chernozems, arable layer: 30–35 cm) and Lukavec (620 m a.s.l., 49°57′ N, 14°99′ E, soil type—skeletic Cambisols, arable layer: 25–30 cm). Basic soil properties according to the fertilizer treatment in 2015 are shown in Table 5.

Table 5. Soil pH, the concentration of P, K, Ca and Mg (mg kg^{-1}) and contents of organic carbon (Cox, %) and total nitrogen (Nt, %) in Caslav, Ivanovice and Lukavec in 2015 (the season before the evaluated period).

	pH	P	K	Ca	Mg	Cox	Nt
Caslav							
Control	6.72	58	114	2858	99	1.26	0.14
FYM	6.55	58	144	2891	118	1.16	0.13
FYM + N2	6.76	76	125	2891	139	1.31	0.16
FYM + NPK2	6.52	188	225	2802	122	1.43	0.17
Ivanovice							
Control	6.85	101	228	4451	200	1.72	0.19
FYM	6.84	171	340	4371	222	1.99	0.23
FYM + N2	6.86	156	320	4481	238	2.13	0.25
FYM + NPK2	6.82	220	438	4215	223	2.04	0.23
Lukavec							
Control	5.93	40	131	1945	92	1.54	0.20
FYM	5.93	56	157	2096	108	1.82	0.23
FYM + N2	5.78	38	164	2047	99	1.86	0.23
FYM + NPK2	5.89	193	207	2011	96	1.82	0.23

The weather conditions in each year (2016–2019), including a comparison with the standard climatological normal (1981–2010), are shown in Table 6. The specific precipitation and average temperatures in each month of the 2016–2019 seasons, including their comparison with the standard climatological normal (1981–2010), are shown in Supplementary Tables S1 and S2. The verbal assessment of the years (Table 6), months and growing seasons (Tables S1 and S2) were done according to [57].

Table 6. The annual sum of precipitation (mm) and the annual mean temperature (°C) compared with the standard climatological normal (1981–2010) in Caslav, Ivanovice and Lukavec (2016–2019).

	Precipitation	Evaluation	Temperature	Evaluation
Caslav				
Normal	593		9.4	
2016	393	V. B. Normal [4]	9.7	Normal
2017	633	Normal	9.6	Normal
2018	318	E. B. Normal [6]	10.8	V. A. Normal [2]
2019	478	B. Normal [4]	10.6	A. Normal [1]
Ivanovice				
Normal	562		9.1	
2016	474	B. Normal [4]	9.9	A. Normal [1]
2017	411	V. B. Normal [5]	9.8	A. Normal [1]
2018	384	V. B. Normal [5]	11.0	E. A. Normal [3]
2019	740	V. A. Normal [2]	10.8	E. A. Normal [3]
Lukavec				
Normal	698		7.8	
2016	601	B. Normal [4]	7.9	Normal
2017	777	A. Normal [1]	7.9	Normal
2018	509	V. B. Normal [5]	8.8	A. Normal [1]
2019	680	Normal	9.1	V. A. Normal [2]

Note: [1] Above Normal; [2] Very Above Normal; [3] Extraordinary Above Normal; [4] Below Normal; [5] Very Below Normal; [6] Extraordinary Below Normal.

The long-term field trials in Caslav, Ivanovice and Lukavec are uniform, so they have the same methodology. There are a total of four fields at each site (Field I, Field II, Field III, Field IV). Each of the four fields is divided into 48 plots of 8 by 8 m. A total of 12 different fertilizer treatments are applied to these plots, each treatment is repeated four times (12 × 4 = 48) in a completely randomized block design. From the total area of the single plot (8 m × 8 m), the central area of 5 m × 5 m is sampled for analyses (elimination of the edge effect).

In this paper, we evaluate four consecutive seasons (2016, 2017, 2018 and 2019) when potatoes (cul. Adéla) were grown. Planting (4800 kg ha^{-1}) was always done in April and harvesting in September. The interline distance was 75 cm. Winter wheat was the pre–crop every year. For a better idea about the experimental design, please take a look at Table 7.

Table 7. The scheme of the trial in the period 2015–2019.

	Field I.	Field II.	Field III.	Field IV.
2015	Winter wheat			
2016	Potatoes	Winter wheat		
2017		Potatoes	Winter wheat	
2018			Potatoes	Winter wheat
2019				Potatoes

For this article, we have selected seven fertilization treatments out of a total of twelve: (1) unfertilized Control (unfertilized since the trial establishment), (2) the cattle farmyard manure (FYM), (3) and (4) FYM applied together with mineral N fertilizers (FYM + N1; FYM + N2), (5), (6) and (7) FYM applied together with mineral N, P and K fertilizers (FYM + NPK1; FYM + NPK2; FYM + NPK3. The specific fertilizer doses in each treatment are shown in Table 8. The rate indicated for mineral fertilizers represents the dosage of net nutrients applied to the field. FYM was applied at the dose of 40 t ha^{-1}. The estimated nutrient content of the FYM is 200, 56 and 236 kg of N, P and K ha^{-1}, respectively.

Table 8. The doses of the nutrients applied in the FYM and individual fertilizer treatments (kg ha^{-1}).

Fertilization Treatment Designation	N	P	K
Control	0	0	0
FYM (40 t ha^{-1})	200	56	236
FYM + N1 (kg ha^{-1})	240	56	236
FYM + N2 (kg ha^{-1})	280	56	236
FYM + NPK1	240	136	336
FYM + NPK2	280	136	336
FYM + NPK3	320	136	336

The mineral N was applied as calcium ammonium nitrate, P as triple superphosphate and K as potassium chloride. The wheat (pre–crop) harvest was followed by moderate stubble tillage. Subsequently, manure was applied to the field in autumn and incorporated into the soil by moderate tillage. The mineral fertilizers N1 (40 kg ha^{-1}), N2 (80 kg ha^{-1}), N3 (80 kg ha^{-1}), P and K were applied during the pre-planting preparation in spring. The remaining 40 kg ha^{-1} N in the N3 treatment (together 120 kg ha^{-1}, Table 8) was applied at the BBCH 16 stage. The FYM and mineral fertilizers were applied manually to the plots.

4.2. Soil Analyses

Following the potatoes harvest, soil samples were taken using the soil probes. The soil samples were taken from the topsoil layer (Caslav and Ivanovice 0–20 cm; Lukavec 0–15 cm). Four samples were taken from each plot. The samples were then mixed and transported to the laboratory for analysis. There, soil samples were dried and sieved. The value of the soil reaction (pH) was determined potentiometrically in 50 mL of 0.2 mol KCl (inoLab pH 730, WTW, Xylem Analytics, Weilheim, Germany). The SOC was determined colourimetrically and by oxidimetric titration according to [58,59]. The soil N content was determined with concentrated sulfuric acid in a heating block (Tecator, Foss A/S, Hillerød, Denmark), followed by the Kjeldahl method [60]. The concentrations of P, K, Mg and Ca were analyzed using the Mehlich III method [61], followed by the ICP—OES analysis (Thermo Scientific ICAP 7400 Duo, ThermoFisher Scientific, Cambridge, UK).

4.3. Data Analyses

For the evaluation of collected data, analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were used using Statistica 13.3 (Tibco Software Inc., Palo Alto, California, USA). In the case of finding the significant differences, Tukey's HSD post hoc analysis was performed. For the evaluation of the relationships between the yields and soil parameters, PCA (principal component analysis) and FA (factor analysis) were used (Statistica 14.0). The linear regression analyses were performed using the QC Expert 3.3 Pro (TriloByte Statistical Software Ltd., Pardubice, the Czech Republic) and NCSS 2019 Statistical Software (NCSS, LLC., Kaysville, UT, USA). The linear regression modelling used the regression triplet, consisting of (1) model design, (2) preliminary data analysis (multicollinearity, heteroskedasticity, autocorrelation and influence points), (3) estimation of parameters using the least square method (LSM) and subsequent testing of the significance of the parameter using the Student's *t*-test, mean square error of prediction, and Akaike information criterion (AIC), (4) regression diagnostics—identification of influence points and verification of the LSM assumptions, (5) construction of the refined model [36]. Statistical significance was tested at a significance level of $p = 0.05$. The weather conditions were analyzed using MS Excel 2007 (Microsoft Corporation, Washington, DC, USA). The analyses, calculations and verbal evaluations were done according to [57], providing the recommendation of the World Meteorological Organization for a description of meteorological or climatological conditions. The weather data were collected from the weather stations running in the nearest vicinity of the field trials and operated by the Czech Hydrometeorological Institute (Prague, Czech Republic).

5. Conclusions

Potato cultivation is significantly influenced by soil and climatic conditions, which primarily affect yields. Suitable soil and climate conditions (lighter soil, higher altitude, higher rainfall, lower temperatures—Lukavec) allow average yields to be achieved, even without the addition of mineral fertilizers. In less suitable conditions (heavier soils, higher temperatures, less rainfall—Caslav, Ivanovice), it depends on the fertility of the soil (soil type) whether it can compensate for the climate deficiencies. The temperature was the parameter strongly and negatively affecting potato yields in our trial (more than precipitation). The occurrence of extraordinary temperatures (Table S2) significantly reduced potato yield, especially in 2018 at all locations. These yield fluctuations (the effect of weather on yields) are and will be encountered more frequently as the occurrence of such affected seasons is predicted to be more frequent.

Manure is a form of fertilizer that significantly increases potato yields, which is an important fact, especially for organic farming. However, without the addition of mineral fertilizers, the modern potato cultivars grown under conventional agriculture practices cannot fully fulfil their yield potential as their requirements for nutrients are higher. The application of manure together with mineral forms of NPK ensures high yields. A dose of 80 kg N ha^{-1} gave comparable yields to a dose of 120 kg N ha^{-1} and represents a reasonable dose in terms of price/performance ratio.

Application of FYM and especially FYM + NPK significantly increased the soil P and K concentrations in Ivanovice and Lukavec, leaving sufficient nutrient reserves in the soil for the upcoming crop. Manure application also slightly (statistically insignificantly) increased the soil pH at all sites but mainly prevents the negative effect of nitrogen fertilizers on lowering the soil pH, which is important information for agriculture that is significantly dependent on mineral nitrogen fertilizers and has long been struggling with a lack of organic manures applied to the soil, as is the case of the Czech Republic.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants10112473/s1, Table S1: The average monthly sum of precipitation (mm) in Caslav, Ivanovice and Lukavec in comparison with the climate normal (1981–2010), Table S2: The average monthly temperatures (°C) in Caslav, Ivanovice and Lukavec in comparison with the climate normal (1981–2010).

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The Effects of Weather and Fertilization on Grain Yield and Stability of Winter Wheat Growing on Orthic Luvisol—Analysis of Long-Term Field Experiment

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Abstract: Based on a long-term experiment in Prague, established in 1954, we analyzed the effect of weather and seven fertilization treatments (mineral and manure treatments) on winter wheat grain yield (GY) and stability. In total, 23 seasons were analyzed, where a wheat crop followed a summer crop of potatoes. A regression analysis showed that, since the experiment started, there has been a significant increase in the annual daily maximum, average, and minimum temperature of 0.5 °C, and an increase in annual rainfall of 0.3 mm. Grain yield was positively associated with April precipitation, mean daily temperature in October, and daily maximum temperature in February. Yields were most stable between years with two fertilizer treatments that supplied a mean of 47 kg N $ha^{-1}yr^{-1}$, 54 kg P $ha^{-1}yr^{-1}$, and 108 kg K $ha^{-1}yr^{-1}$. The rate of N at which grain yield was optimized was determined according to the linear-plateau (LP) and quadratic response models as 44 kg N $ha^{-1}yr^{-1}$ for the long-strawed varieties and 87 kg N $ha^{-1}yr^{-1}$for short-strawed varieties. A gradual increase in yields was observed in all treatments, including the unfertilized control, which was attributed to improved varieties rather than to a changing climate.

Keywords: *Triticum aestivum* L.; temperature; precipitation; mineral fertilizers; farmyard manure; non-linear response models; climate change

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1. Introduction

Wheat is the most widely grown cereal in the EU, withan estimated production of 130 million tonnes in 2021. Wheat production, grain yield (GY), and its quality areinfluenced by a wide range of variables, mainly by the soil–climate conditions and fertilization. Other factors, such as the preceding crop in the crop rotation [1,2], wheat variety [3], or tillage practices [4], also play a significant role in yield formation and stability.

The effect of weather on agricultural productivity has been analyzed for a long time [5]. In the context of weather, climate change is currently the most highlighted term. Its precise definition can be problematic [6], but generally, it refers to long-term climate shifts in temperature and precipitation patterns, directly affecting short-term interannual weather variation (more frequent occurrence of extraordinary weather circumstances during the growing seasons). Climate change is a long-term and gradual process. There is a wide range of papers analyzing the impacts of ongoing climate change on future agricultural production based on different models. These papers, analyzing the relationship between changing climatic conditions and crop production, have been published since the early 1990s [7–9] and continue to this day, confirming that climate change is significantly affecting and will continue to affect crop yields and quality, positively or negatively (according to the locality), and that different regions will be affected to a different extent [10–12]. In England, for example, conditions for growing wheat can be expected to remain favorable until the middle of the 21st century, without the long periods of drought that are, on the other hand, expected in some European countries [13]. In Spain, conditions for growing wheat can be expected to remain favorable, while conditions for sunflower are likely to deteriorate in the future due to climate change [14]. In the Czech Republic, climate change is connected with more frequent occurrence of intensive dry episodes [15]. For example, a severe drought was recorded in the 2011–2012 season in south Moravia region, resulting in the lowest cereal yields in the past 52 years [16,17]. Severe wheat yield losses were also recorded in France, Belgium, and Switzerland in 2016, when mean wheat yields decreased significantly in comparison with the previous ten years due to either drought or abnormally wet conditions [18]. The exact impact of climate change on crop production is difficult to assess. Long-term field trials can provide some insight, as they are well defined, include different and controlled fertilizer treatments, and detailed weather observations are made at the trial site. Fertilization represents one of the tools that help balance the effects of unpredictable and rapidly changing weather conditions.Thanks to data from long-term experiments, we can analyze the effect of weather in each month on crop yields, the effect of different fertilizer regimes on yield and its stability, and the recommend optimal fertilizer rates for selected soil–climate conditions. A study of a long-term experiment at Müncheberg (northeast Germany) showed that the effect of weather on spring barley yields was major, accounting for 55% of variation, so yields are strongly dependent on the weather conditions of the year. The Bayesian linear regression analysis showed that yield was influenced mostly by rainfall between April and July, especially for treatments fertilized with high rates of mineral N. Fertilizer application had a much smaller effect on yield, accounting for 11% of variation. Mineral N application also reduced year-to-year variation in yield [19]. A similar study of long-term effects of weather and fertilization on the yields of winter wheat at Rothamsted showed a correlation between winter wheat yield and mean temperature in November, April, and May, and precipitation in October, February, and June. Yields of spring barley were correlated with mean temperature between February and June, and with precipitation from April to July and in September [20]. An analysis of two long-term experiments in Germany showed that barley grown in crop rotations dominated by cereals proved a yield with lower stability and higher production risk than rotations with higher crop diversity. The highest yield stability of barley was reached with a medium N rate (70 kg N $ha^{-1}yr^{-1}$) [21,22].

Inspired by the above-mentioned papers, we decided to analyze the long-term field experiment (LTE) in Prague, established in 1954. The aim of our work is to analyze whether the yield of winter wheat is controlled by weather conditions, which month conditions (max. temperature, mean temperature, min. temperature, precipitation) significantly influence the yield, what type of fertilization provides stable yields, and what fertilizer dose represents the optimum under given soil and climatic conditions.

2. Results

2.1. Yield Development

Winter wheat GY increased since the start of the LTE for all fertilizer treatments, including the unfertilized control. The average seasonal GY increase for control, FYM, NPK1, NPK2, NPK3, NPK4, and FYM+NPK was approximately 24, 35, 40, 43, 47, 53, and 63 kg ha^{-1}, respectively (Figure 1a). As the short-strawed wheat varieties began to be grown in 1983, the straw yield (STY) has been declining (Figure 1b).

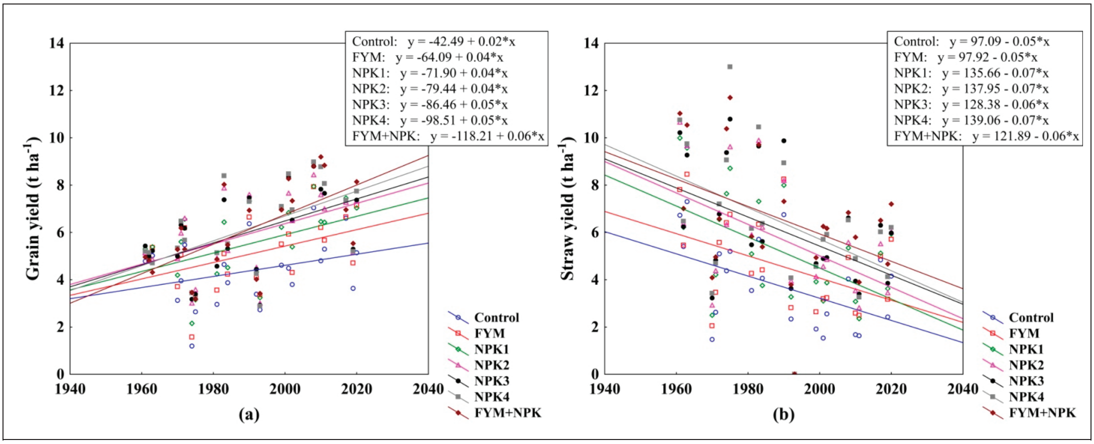

Figure 1. The development of (**a**) grain yield, GY (t ha^{-1}) and (**b**) straw yield, STY (t ha^{-1}) from 1961 to 2020, as affected by the fertilizer treatment.

2.2. Weather Conditions

The average daily maximum temperature averaged over the years increased by 0.05 °C from 1954 to 2020, while the average daily temperature increased by 0.05 °C, minimum temperature by 0.06 °C, and precipitation increased by 0.3 mm yr^{-1} (Figure 2). In summary, the site of the LTE has been gradually warming since 1954, and the amount of rainfall increased slightly.

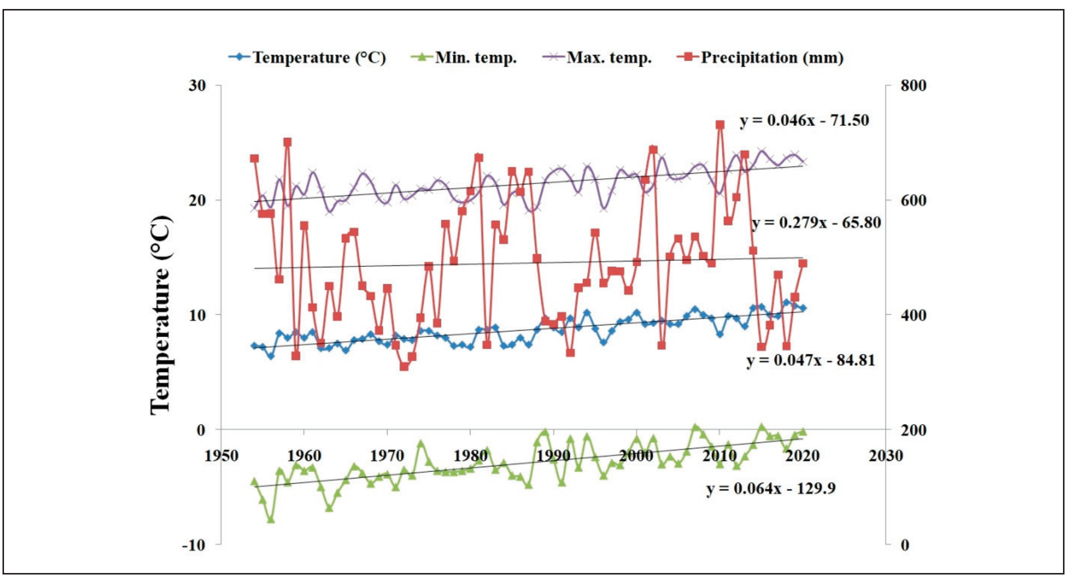

Figure 2. The development of average, min., max. temperatures and precipitation, LTE Prague, 1961–2020.

An equivalent analysis of growing seasons for which there were corresponding wheat yield data analyzed in this paper (1961 to 2020) also showed similar trends. The daily maximum, average, and minimum temperatures increased by 0.05 °C, 0.04 °C, and 0.06 °C, respectively, while precipitation increased by 1.6 mm yr^{-1} (Figure 3).

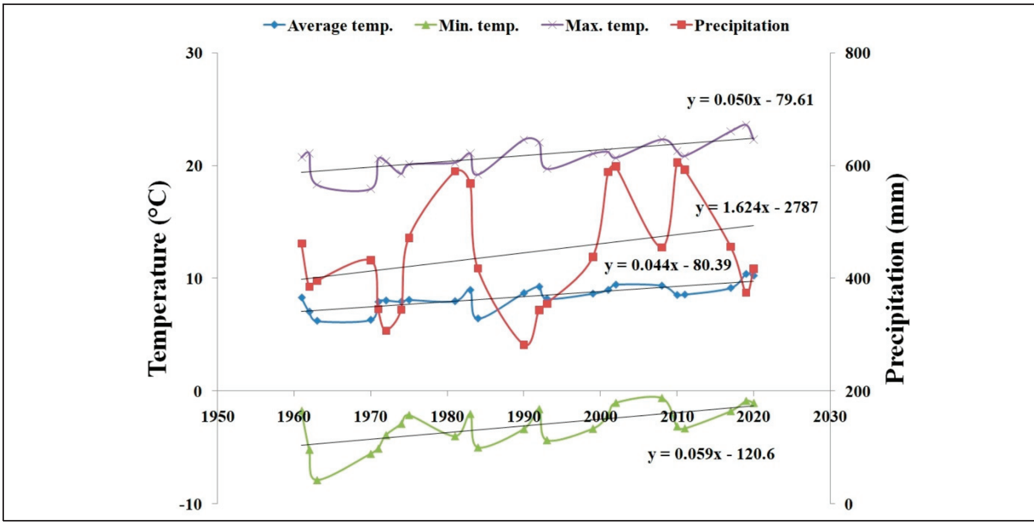

Figure 3. The development of average, min., max. temperatures and precipitation, LTE Prague, 1961–2020 (data from 23 seasons that are analyzed in this paper).

2.3. Relationship between Weather and Yields

To answer the question of whether the weather differed significantly between periods with different wheat varieties, we used ANCOVA, where GY, STY, temperature, and precipitation served as covariates. According to the results, the temperature did not differ significantly between the periods when each variety was grown ($d.f. = 8$, $F = 2.2$, $p = 0.114$). In the case of precipitation, we observed a significant difference ($d.f. = 8$, $F = 2.9$, $p < 0.05$) between the period when the Zdar variety was grown (lowest mean precipitation, 327 mm, mean value over three seasons, 1990, 1992, and 1993) and the period when the Alka variety was grown (highest mean precipitation, 593 mm, mean value over two seasons, 2001 and 2002). These two periods were the only ones significantly different (in terms of rainfall). There were no significant differences in rainfall between the other periods. From this point of view, the general effect of increasing temperatures and precipitation on gradually increasing yields is so far insignificant, and the increasing trend in GY is mainly due to the use of modern wheat varieties. The conditions of a particular year affect yields significantly. We used multiple linear regression (MLR) and correlation to investigate the relationship between the weather and yield. The MLR answers the question of whether yields can be predicted from the temperature or rainfall in a particular month of the growing season. We evaluated the relationship between the rainfall, max., average, and min. temperatures and between the average GY (all fertilizer treatments together) and the GY of each fertilizer treatment (separately—control, FYM, NPK1...FYM+NPK). The results of the MLR were not statistically significant in any case (n = 32), as the p value ranged from 0.07 to 0.9. On the other hand, the results of the correlation analysis were already significant. In the case of rainfall, we found a positive significant moderate correlation between GY and rainfall in April for all fertilizer treatments (r = 0.5). According to Figure 4a, the highest wheat yields can be expected when 50 to 60 mm of rain falls in April. There was also a significant positive correlation between GY and average temperature in October (r = 0.5, positive moderate correlation, Figure 4b) and maximal temperature in February (r = 0.6, positive moderate correlation, Figure 4c). Higher yields were therefore associated with wetter conditions in April up to a maximum of 60 mm month^{-1} and warmer conditions in October and February. However, the quadratic function in Figure 4b is approaching its maximum, and a further increase in temperature could lead to a decrease in yield in the future.

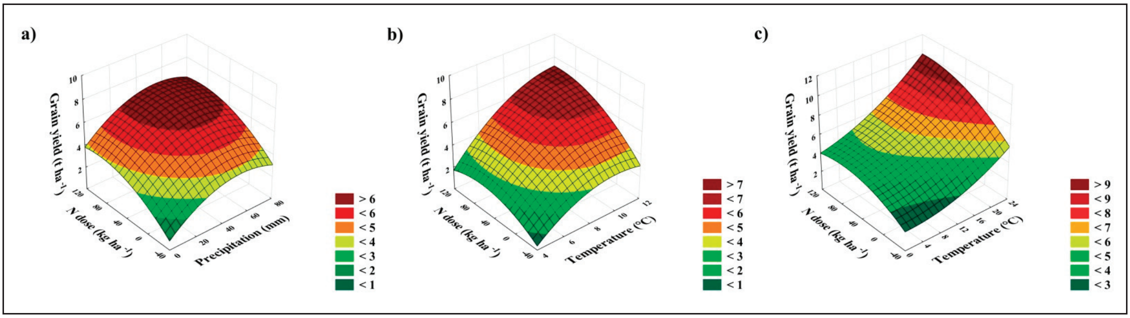

Figure 4. Response surface of the effect of applied N (kg ha^{-1} N) on winter wheat GY (t ha^{-1}) as affected by (**a**) April rainfall, (**b**) average October temperature, (**c**) max. February temperature.

2.4. Yield Stability

According to Kang's ranksum statistics, in which Shukla's stability variance was used to identify high-yielding and stable fertilizer treatments, the highest stability of long-strawed wheat varieties was provided by the NPK3 treatment (rank 1), followed by NPK2 (rank 2), NPK4 (rank 3), NPK1 (rank 4), FYM+NPK (rank 5), FYM (rank 6), and control (rank 7) treatments. For the short-strawed wheat varieties, the most stable yields were provided by the NPK2 and NPK3 treatments (rank 1), followed by NPK 4 (rank 3), FYM+NPK (rank 4), NPK1 (rank 5), FYM (rank 6), and control (rank 7) treatments. Treatments with rank 1 are the most desirable.

2.5. Effect of Fertilization on Grain Yield—N Dose Optimization

According to the ANCOVA results (STY as covariate), the GY of long-strawed wheat varieties was significantly affected by fertilizer treatment (d.f. = 6, F = 6.4, $p < 0.001$). The productivity of short-strawed wheat varieties was also significantly affected by fertilizer treatment (d.f. = 6, F = 6.6, $p < 0.001$) (Table 1).

Table 1. The effect of fertilizer treatments on GY (t ha^{-1}) of long- and short-strawed wheat varieties.

	Long-Strawed Var.	**Short-Strawed Var.**
	GY (t ha^{-1})	
Control	3.8 ^{A}	4.7 ^{A}
FYM	4.3 ^{AB}	5.5 ^{B}
NPK1	4.6 ^{B}	6.1 ^{BC}
NPK2	4.9 ^{B}	6.6 ^{CD}
NPK3	4.9 ^{B}	6.7 ^{CD}
NPK4	4.9 ^{B}	6.9 ^{D}
FYM+NPK	4.7 ^{B}	7.0 ^{D}

The capital letters following the average GY present the result of the post hoc analysis (Tukey's HSD test). Average GY with the same letter are not statistically significantly different from each other.

In the case of long-strawed varieties, the lowest GY was recorded in the control. The application of FYM to the preceding crop, compared to the control, resulted in a slight increase in GY, while no statistically significant difference was observed between FYM and the other fertilized treatments (Table 1). For short-strawed wheat varieties, the unfertilized control also provided the lowest GY, while application of the FYM to the preceding crop resulted in significantly higher GY (+800 kg ha^{-1} in comparison with control). The highest GY was provided by the FYM+NPK treatment, but no statistically significant difference was recorded between NPK2 and FYM+NPK treatments (Table 1).

For the purpose of fertilization optimization, two non-linear models were used: the linear-plateau (LP) and quadratic models. For long-strawed varieties (1961–1981, nine seasons), the shoulder point of the LP model occurred at a dose of 15 kg ha^{-1} N, corresponding with a yield of 4.8 t ha^{-1} (Figure 5, left). According to the quadratic model, the maximal yield occurred at a dose of 73 kg ha^{-1} N, corresponding with a yield of 4.9 t ha^{-1} (Figure 5, right, y = $-0.0002x^2 + 0.0278x + 3.9106$). As the results of the LP model can be considered too conservative, and the results of the quadratic model as redundantly high (from the point of view of efficiency, economy, and environment protection), a reasonable recommendation for the optimum N rate should be based on the results of both models (the average value) [23]. In our case, the optimum N dose for the long-strawed wheat varieties occurs at 44 kg ha^{-1} N.

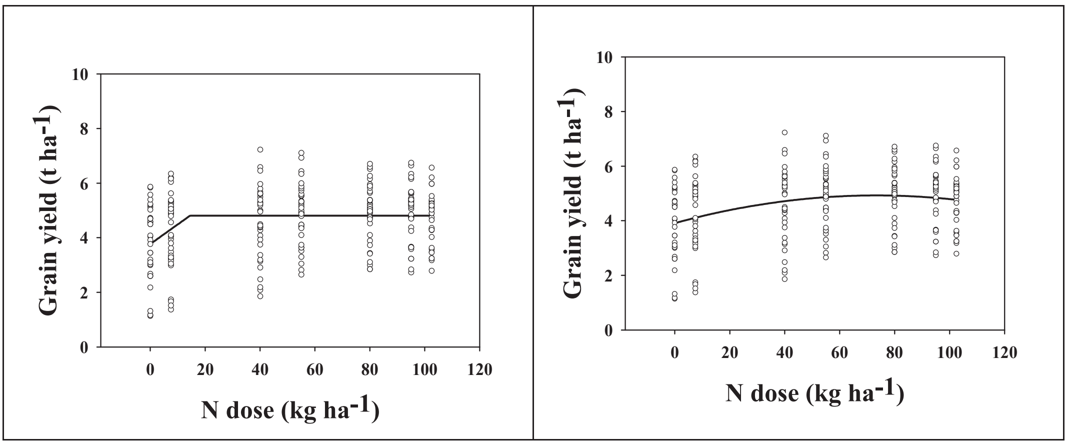

Figure 5. Grain yield (t ha^{-1}) of long-strawed varieties as affected by N dose (kg ha^{-1}). The data come from 9 seasons (1961–1981). (**Left**) data interleaved with the linear-plateau model. (**Right**) data interleaved with the quadratic model. The shoulder point of the LP model (**left**) occurred at the dose of 15 kg ha^{-1} N (4.8 t ha^{-1}). According to the quadratic model (**right**), the maximal yield occurred at the dose of 73 kg ha^{-1} N (4.9 t ha^{-1}).

The introduction of short-strawed wheat varieties resulted in a significant increase in grain yield (ANCOVA, straw yield as a covariate factor, $p < 0.001$). The average grain yield of long-strawed varieties (1961–1981, 9 seasons) was 4.6 t ha^{-1}, while the average grain yield of short-strawed varieties (1983–2020, 14 seasons) was 6.2 t ha^{-1}.

According to the ANCOVA results (Table 1), the NPK2 treatment (55 kg ha^{-1} N) provided comparable grain yield as the FYM+NPK treatment (102.5 kg ha^{-1} N). The application of the FYM to the preceding crop resulted in GY averaging 800 kg ha^{-1} higher than the control, and the difference was significant. The combined application of FYM and NPK resulted in slightly higher yield (+100 kg ha^{-1}) than with the NPK4 treatment, but the difference was not significant.

According to the LP model, the shoulder point occurred at the dose of 66 kg ha^{-1} N, corresponding with a GYof 6.9 t ha^{-1} (Figure 6, left). According to the approximation of the quadratic model (Figure 6, right, y = $-0.000171x^2 + 0.0367x + 4.974$), the average maximal yield (the local maximum of the quadratic function) was reached at the dose of 107 kg ha^{-1} N, corresponding with a yield of 7.0 t ha^{-1}. As mentioned above, this dose of mineral N is redundantly high, and the recommended dose of mineral N for short-strawed varieties grown under comparable soil–climate conditions, as in the LTE, should be approximately 87 kg ha^{-1} (the mean value between 66 and 107 kg ha^{-1} N). This value also provides the most stable GY.

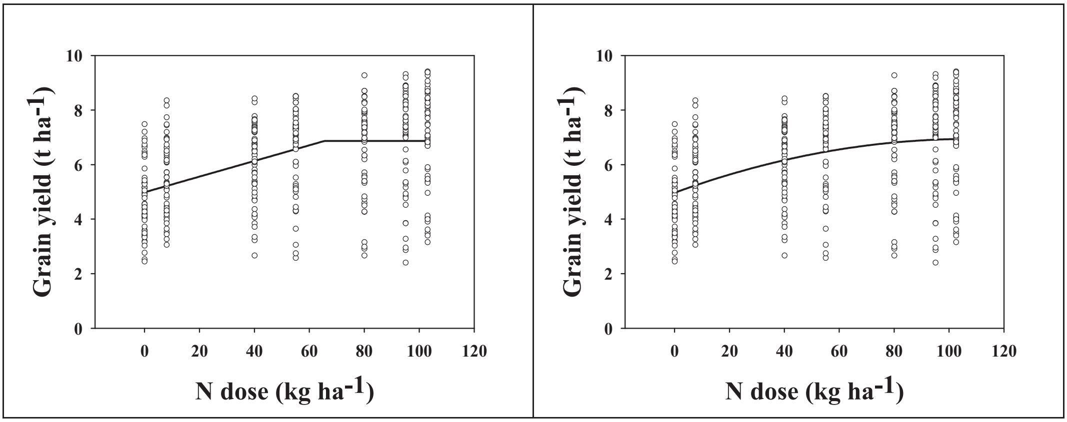

Figure 6. Grain yield (t ha^{-1}) of short-strawed wheat varieties as affected by N dose (kg ha^{-1}). The data come from 14 seasons (1963–2020). (**Left**) data interleaved with the linear-plateau model. (**Right**) data interleaved with the quadratic model. The shoulder point of the LP model (**left**) occurred at the dose of 66 kg ha^{-1} N (6.9 t ha^{-1}). According to the quadratic model (**right**), the maximal yield occurred at the dose of 107 kg ha^{-1} N (7.0 t ha^{-1}).

3. Discussion

3.1. The Effect of the Weather on GY

Based on the weather analysis, we can say that the conditions at the LTE site are gradually changing. We observed an increase in maximum, average, and minimum temperature as well as precipitation during the year and season for winter crops. In the case of temperatures, this trend is evident throughout the Czech Republic. According to Zahradníček [24], each decade is generally warmer than the previous period, with enhanced warming between 2011 and 2019. In the case of total precipitation, the long-term analysis published by Brázdil [25] proved that the fluctuation of total precipitation is stable in the Czech Republic; negative precipitation trends were recorded between April and June, while positive trends were recorded between July and September. In our paper, we analyzed the relationship between GY and weather parameters of each month of the growing season via the MLR and correlation. The same approaches have been used for analyses of long-term experiments in Müncheberg (Germany) [19] and Rothamsted (UK) [20]. According to the linear regression model used in Germany, the total precipitation during April and July was positively correlated with spring barley GY (when high doses of mineral N were applied), while a negative relationship was found for precipitation in March and temperature in April. In our case, the MLR provided no significant results (n = 32). This comparison is for illustrative purposes only. The weather conditions and weather development throughout time are different at each location and are, therefore, site–specific. The results of one site cannot be generalized to other sites with different soil and climatic conditions. According to the result from Rothamsted [20], the wheat GY is sensitive to mean temperature in November, April, and May, and to precipitation in October, February, and June. Based on the results of this work, the optimum mean November temperature ranges between 6 °C and 7 °C. The April temperature between 8 and 8.5 °C maximizes the wheat GY, while a lower precipitation in June leads to lower yields [20]. In our case, we found a positive relationship between April precipitation and GY, with the optimum ranging between 50 and 60 mm (Figure 4a). Higher or lower precipitation will result in lower than optimum yields. As the mean April precipitation is approximately 32 mm (1954–2020), and the trend of April precipitation is decreasing [25], we can expect that the negative effect of April rainfall on wheat GY will be enhanced in the future. The mean temperature in October was also positively correlated with yields (Figure 4b). Higher October temperatures will beneficially influence seedling emergence and plant germination and provide time for development of stronger plants.

Finally, the increasing temperature in February was also evaluated as a factor positively affecting wheat GY (Figure 4c), allowing earlier start of the plant development, provided that there are no other negative aspects, such as higher incidence of pests, diseases, soil acidification, etc.

3.2. Yield Stability

The most stable yields of long-strawed wheat varieties (1961–1981) were provided by treatments from the middle range of mineral fertilizers (NPK2 and NPK3, 55–80 kg ha^{-1} N), i.e., neither the lowest nor the highest treatments. The old long-strawed wheat varieties had lower yields than short-strawed varieties [26–28] due to better resistance to lodging and diseases, earlier reaching of anthesis, and a longer grain filling period [29]. In the case of short-strawed wheat varieties, the results were the same; the highest yield stability was provided by the same treatments (NPK2 and NPK3). Application of higher doses of mineral N (such as NPK4) generally resulted in slightly higher yields, but according to results of yield stability, also in higher year-to-year variation, i.e., lower stability.

The unfertilized control had the lowest yield stability in both long- and short-strawed varieties. Although the average yields of unfertilized controls increase over time, the wheat in these plots is mainly dependent on the pre-crop effect in terms of nutrients (edge effects were eliminated in the experiment by the harvesting methodology; only the central area of the plot was harvested for experimental purposes). This is confirmed in a study of the long-term experiment at Giessen University (Germany) [22], where similar results were found for an unfertilized control treatment and a similar pattern for PK treatment (without mineral N), showing an unprecedented effect of mineral N on yield stability. Analysis of the long-term experiments in Germany also showed a low ability of unfertilized treatments or treatments without mineral N to provide stable yields [30]. According to this study, the yield stability was also negatively affected by omission of mineral potassium, which can support yield stability, especially under ongoing climate change, as this element controls water management in the plant.

Application of manure alone (without mineral fertilizers—FYM treatment) to the preceding crop resulted in the second lowest yield stability (in both long- and short-strawed varieties). Similar results were published by Macholdt [31]. We assume that the vast majority of the nutrients from FYM, released by the mineralization, were utilized by the preceding potato crop. The rest of the nutrients, together with the expected beneficial effect of FYM on soil parameters [32,33], slightly (and significantly, in short-strawed varieties) increased the GY (when compared to control), but the fluctuation was still high.

3.3. The Effect of Fertilization on GY

As mentioned above, the effect of FYM application to the preceding crop influenced wheat GY. For the long-strawed varieties, there was no significant difference between FYM and control, although FYM application resulted in slightly higher yields. For the short-strawed varieties, the effect was significant (Table 1). Manure not only provides nutrients but also has a beneficial effect on soil properties, positively influencing soil microbial fauna and its activity, nutrient turnover, soil organic carbon, and nitrogen content [33,34], and the effect on wheat GY is visible even three years after its application [35]. This synergy (nutrients + effect on soil) is behind why we observed a significant difference in the short-strawed varieties when comparing FYM versus control. Long-strawed varieties had lower overall yield than short-strawed varieties and were less responsive to N because of their lower nutrient requirements (even high fertilizer rates did not significantly increase yields—quite the opposite, as confirmed by LP and quadratic response models). The introduction of short-strawed wheat varieties into the experiment was associated with higher yields but also with higher nutrient requirements. The quantity of nitrogen to achieve optimum yields in short-strawed varieties was about double that of long-strawed varieties (Figure 5), as the recommended dose of mineral N increased from 44 kg ha^{-1} N to 87 kg ha^{-1} N. These recommended doses were calculated according to Hochmuth [23], based on two non-linear models analyzing the response of GY to N doses. These recommendations are site–specific and should be calculated in relation to the soil and climate conditions. Other researchers have published their own recommendations according to the soil–climate conditions of the analyzed experiments [20,36–39]. Optimum fertilizer application is important for two reasons. Firstly, it must be economic, so that a positive economic return is achieved for the investment in a fertilizer. Secondly, it must be environmentally friendly, as excessive fertilizer application is not only wasteful but also negatively affects the quality of the environment [40,41].

4. Materials and Methods

4.1. Site Description

The LTE in Prague can be found on the western edge of the city of Prague, the Czech Republic, central Europe, 50°05′15″ N, 14°17′28″ E. According to the Köppen–Geiger climate classification [42], the site is located in a warm-summer continental climate area (Dfb). The LTE in Prague was established in 1954 with the aim of measuringthe long-term effect of different fertilizer treatments (mineral and organic fertilizers, organic manures), crop rotations, and weather conditions on the yield and quality of arable crops and soil properties. The soil type is Orthic Luvisol, formed by diluvial sediments mixed with loess [43]. The topsoil depth is approximately 0.3 m. The standard climatological long-term mean temperature is 8.7 °C, and the precipitation is 490.4 mm (1954–2020, Crop Research Institute meteorological station). The altitude of the site is 370 m a.s.l.

4.2. The Long-Term Experiment (LTE) Description

The LTE in Prague is a large-scale field experiment, consisting of five fields (fields I., II., III., IV., and B). The size of one field is 144 × 96 m (cca 1.4 ha). Each field is divided into 96 individual plots. The size of one plot is 12 × 12 m. In each field, the effects of 24 fertilizer treatments are measured, each treatment replicated four times (24 × 4 = 96 plots) in a completely randomized block design. The crop rotation of the fields II., III., and IV. (analyzed in this paper) consists of nine crops, growing in the order alfalfa, alfalfa, winter wheat, sugar beet, spring barley, potatoes, winter wheat, sugar beet, and spring barley. Out of 24 fertilizer treatments, 7 are analyzed in this paper: (1) control (unfertilized since the LTE establishment), (2) farmyard manure (FYM), (3) NPK1, (4) NPK2, (5) NPK3, (6) NPK4, and (7) FYM+NPK (FYM+NPK4 specifically, but the FYM+NPK abbreviation is used in the paper). The doses of mineral fertilizers are: NPK1—40, 48, 96 kg ha^{-1}; NPK2—55, 60, 120 kg ha^{-1}; NPK3—80, 48, 96 kg ha^{-1}; NPK4—95, 60, 120 kg ha^{-1}. The mineral N is applied as limestone ammonium nitrate, P as super phosphate, and K as potassium chloride. Mineral fertilizers are spread evenly by hand. The cattle farmyard manure in the FYM and FYM+NPK treatments is applied to the preceding crop, potatoes, at a dose of 15 t ha^{-1} (approximately 75 kg N). As winter wheat is the first crop following, the estimated amount of N available to and utilizable by wheat represents 10%—7.5 kg ha^{-1}. From this point of view, the FYM treatment is calculated as a dose of N of 7.5 kg ha^{-1} and the FYM+NPK treatment as a dose of 102.5 kg ha^{-1} N.

In this paper, we analyze 23 seasons in total, when winter wheat followed by potatoes are grown. These seasons are (the year of harvest) 1961, 1962, 1963, 1970, 1971, 1972, 1974, 1975, 1981, 1983, 1984, 1990, 1992, 1993, 1999, 2001, 2002, 2008, 2010, 2011, 2017, 2019, and 2020. Different wheat varieties have been grown in the experiment since 1961. The long-strawed varieties are Pyšelka (1961–1963), Mironovská (1970–1972), and Jubilar (1974–1981). The short-strawed varieties are Juna (1983–1984), Zdar (1990–1993), Samanta (1999), Alka (2001–2002), Barroko (2008–2010), and Mulan (2011–2020). The sowing of the winter wheat occurs in October; the depth of sowing usually ranges from 3 to 4 cm, and the distance between rows is 0.125 m. Pesticides are used if necessary, and growth regulators are never applied.

4.3. Data Analysis

For the calculation of correlations, linear and multiple linear regressions, analysis of covariance (ANCOVA), post hoc analyses (Tukey's HSD test), and graphical outputs, Statistica 14.0 was used (TIBCO Software, Palo Alto, CA, USA). For the calculation of linear-plateau and quadratic models, SigmaPlot 14.5 was used (Systat Software Inc., Chicago, Illinios, USA). The analysis of stability was performed using the StabilitySoft software [44].

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Article

Does Elemental Sulfur Act as an Effective Measure to Control the Seasonal Growth Dynamics of Potato Tubers (*Solanum tuberosum* L.)?

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Abstract: The in-season dynamics of potato tuber biomass (TTB) growth requires effective nitrogen (N) control. This hypothesis was tested in 2006 and 2007. The two-factorial experiment with two rates of N (60, 120 kg ha^{-1}) and sulfur (S; 0, 50 kg ha^{-1}) was carried out in the split-plot design. The third factor was the sampling of plants at 10-day intervals. The collected plant material was divided into leaves, stems, stolons + roots, and tubers. The seasonal trend of TTB was linear, while the biomass of leaves, stems, and stolons + roots was consistent with polynomial regression models. TTB was controlled by (i) the date of potato growth after emergence, when the TTB exceeded the leaf biomass (DAE_{crit}); (ii) the stem growth rate; and iii) the share of stems in the total potato biomass. TTB growth was reduced when DAE_{crit} preceded the DAE_{op} for leaf biomass, determining its maximum. This phenomenon appeared in 2007 on plots fertilized only with N. The absolute growth rate of the stem biomass, exceeding $\frac{1}{4}$ of that of the tuber biomass in the descending phase, resulted in an increased and prolonged share of stems in the total potato biomass, which ultimately led to a decrease in tuber yield. The use of sulfur to balance the N, applied effectively, controlled the growth rate of potato organs competing with tubers.

Keywords: growth dynamics; dry matter partitioning; tuber vs. non-storage organs competition; nitrogen rates; elemental sulfur

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1. Introduction

Plant growth depends on the net assimilate production by its photosynthetic tissues, and on their subsequent partitioning between other assimilate-dependent and actively growing tissues [1,2]. The capacity of plant tissues to produce the assimilates is expressed as its source strength, which is a result of the size of the source and its activity. Sink tissues consume the assimilates provided by the plant source mainly for growth. The sink capacity of plant tissues to reuse assimilates is defined as the sink strength, which is determined by the size of the sink and its activity [2].

There is no doubt that potato tubers are a net sink and mature leaves are the pure source organ [3]. The growth continuity of potato organs undergoes change as a result of the transformation of the underground stems, i.e., stolons into tubers. This process, driven by plant hormones, is, however, sensitive to external factors like temperature, soil moisture, and the supply of nutrients [4]. The availability of N to the potato does not affect the number of leaves but increases their surface area [5]. Deficiency in two key growth factors, i.e., water and N, is the main reason for source strength disturbance, which subsequently has a negative effect on the production of assimilates. The in-season variability in the quantity of produced assimilates impacts the structure of their subsequent partitioning between plant tissues [6].

An efficient strategy to increase potato yield, as suggested by Katoh et al. [7], consists of two steps. Firstly, the growth processes taking place in the period extending from the early growth stage to the tuber-bulking stage should lead to the intensification of assimilate production in the source tissues. The intensification of the source activity can be achieved as a result of its higher net photosynthetic activity by increasing the leaf surface area. Secondly, the growth processes should be oriented towards increasing the size of the tubers in the early bulking phase. This concept has, however, some weak points. The key target can only be achieved as long as there is an adequate and balanced supply of N [8–10]. The targets of other nutrient applications are to increase both N-use efficiency (NUE) and the rate of assimilates transported to the growing tubers [11,12]. Enhancement of the source activity based only on the N dose may disturb the processes responsible for tuber initiation [4].

Engels and Marschner [13] stated that the final tuber yield depends on the size and growth rate of young tubers. The potential of the juvenile potato tuber for assimilate absorption depends, however, on its N content [14]. The realization of the tuber production potential depends first of all on the supply of potassium (K) [11,15]. Synchrony between the photosynthetic activity of leaves and tuber growth requires at least a good supply of other nutrients, such as K, magnesium (Mg), and phosphorus (P) [1,11,16]. K and Mg are responsible for the transport of assimilates in the phloem from the leaves to the enlarging tubers [16,17]. K shortage leads to a tremendous loss of yield, irrespective of the primary N status in young tubers [14].

The N supply to the potato during the growing season can be effectively controlled by a sound use of other agronomic measures. The basic one is regulation of the N dose. It is well-documented that too low an N supply to potatoes in the period of its early growth may result in a reduction of the photosynthetic area of the plants. On the other hand, too high an N rate may disturb plant growth and the processes of potato tuberization [4,18,19]. Another agronomic solution focused on the effective control of the N supply is the use of elementary S (S^0). It has been well-documented that S^0 oxidation is mediated by soil bacteria. The end products are a sulfuric ion (SO_4^{2-}) and H^+ [20]. Potatoes require a good supply of S for effective growth and to lower the soil pH, especially in soils with a high pH. In conditions of good S supply, a simultaneous increase in the efficiency of N can also be expected [21,22].

White et al. [6] concluded in an extensive review that crop productivity is driven by growth limitations imposed by the degree of source and sink balance during development. The potato tuber is the pure sink tissue of this crop. All other tissues begin their life cycle as sink organs [7]. Thus, the following key question arises: "Is the tuber sink strength limited by the strength of other potato sinks or vice versa?" Further minor questions are as follows:

1. Does the pre-tuberization period of potato growth impact the development of tuber sink strength?
2. To what extent and for how long do the temporary sinks limit the tuber sink strength?
3. Is it possible to effectively control the sink strength of potato tissues competing with the tubers by agronomic measures?

The objective of the study was to (i) quantify the seasonal growth trends in the biomass of potato organs competing with tubers and (ii) evaluate the impact of elemental sulfur on the in-season relationships between the biomass of potato organs.

2. Results

2.1. In-Season Trends of Tuber Yield Development

The distinctly different annual growth patterns of TTB response to experimental factors were the basis for the analysis of tuber yield growth trends for each study year separately. In 2006, TTB responded significantly to the N × T interaction, and indeed to T (sampling interval (Table 1, Figure 4a). The general trend of potato tuber yield development during the growing season was as follows:

$$TTB = 22.49\ DAE - 661.2 \text{ for } R^2 = 0.99,\ n = 8, \text{ and } p \leq 0.01 \quad (1)$$

Table 1. Means of response variables and significance of F values for fixed sources of variation during the potato growth season.

Source of Variation	Factor Level	Degree of Freedom	Roots and Stolons	Leaves	Stems	Tubers	Total Biomass	Tuber's Harvest Index	Roots and Stolons	Leaves	Stems	Tubers	Total Biomass	Tuber's Harvest Index
			g m^{-2} DW					%	g m^{-2} DW					%
			2006						2007					
Nitrogen (N) kg ha^{-1}	60	1	48.2	334.6	304.9	910.9	1385.9	48.6	51.7	245.8	414.0	861.6	1359.4	47.2
	120		49.3	312.7	298.3	915.5	1361.5	49.1	56.3	282.4	490.1	721.0	1356.6	39.2
F value and significance			2.3 ns	6.8 **	0.9 ns	0.1 ns	2.1 ns	0.7 ns	29.3 ***	43.8 ***	29.3 ***	43.8 ***	68.3 ***	94.9 ***
Sulfur (S) kg ha^{-1}	0	1	48.4	333.3	302.6	897.6	1371.6	48.3	54.7	263.3	463.7	686.8	1285.3	39.8
	50		49.3	314.0	300.6	914.5	1375.8	49.4	53.4	265.4	440.3	896.0	1430.7	46.6
F value and significance			0.84 ns ‡	5.3 *	0.1 ns	6.8 **	0.06 ns	4.5 *	2.3 ns	0.1 ns	2.3 ns ‡	0.1 ns	6.4 **	65.6 ***
Sampling time (T), DAE [1]	15	9 x	17.9	51.2	nd †	nd	69.1	nd	7.7	71.4	nd †	nd	79.1	nd
	25	8 y	36.1	187.4	61.9	nd	285.4	nd	49.5	282.9	210.3	nd	542.6	nd
	35	7 z	42.6	308.6	131.9	73.4	556.5	16.0	40.2	388.1	355.6	158.0	941.9	13.2
	45		63.0	396.5	247.1	400.5	1107.0	23.0	58.1	381.6	421.4	277.4	1138.5	35.9
	55		64.9	452.6	384.7	604.9	1507.0	30.8	62.7	325.5	493.6	426.1	1307.9	41.0
	65		61.5	498.6	429.7	770.2	1759.9	40.0	75.0	280.6	575.6	645.9	1577.1	43.8
	75		60.3	485.5	456.5	1027.3	2029.6	46.0	76.6	268.6	593.3	828.4	1766.9	50.4
	85		52.2	349.8	389.5	1269.6	2061.0	54.9	68.3	247.8	555.0	1087.5	1958.6	61.9
	95		47.3	300.4	320.9	1469.0	2134,5	62.9	56.3	204.9	489.3	1299.3	2049.8	69.0
	105		41.9	206.1	292.2	1686.7	2226.9	72.0	46.0	189.8	374.2	1608.0	2218.1	75.8
F value and significance			160.0 ***	117.5 ***	162.3 ***	1136.1 ***	930.0 ***	671.2 ***	222.6 ***	116.3 ***	222.6 ***	116.3 ***	81.0 ***	277.7 ***
Significance of interactions ‡														
N × S		1	*	***	***	ns	***	***	ns	ns	ns	***	***	***
N × T		9 x,8 y,7 z	***	ns	***	*	**	*	ns	***	**	ns	ns	ns
S × T		9,8,7	***	ns	ns	ns	ns	ns	***	*	**	**	**	ns
N × S × T		9,8,7	***	***	***	ns	***	***	**	ns	ns	ns	ns	ns

‡ ns = Non-significant at $p \leq 0.05$; *, **, ***; significant at $p \leq 0.05$, 0.01, 0.001,. † Non-determined; x denotes roots + stolons, leaves, total biomass; y stems; z tuber dry weight and; z also tuber harvest index. Within a column, means followed by same lowercase letter are not significantly different at $p \leq 0.05$. Letters are not shown for significant main effects that also had a significant interaction effect. ‡ Means comparison for variables having a significant interaction effect are shown in Figures 1–4 or discussed in text. Legend: DAE [1]—Days after Emergence.

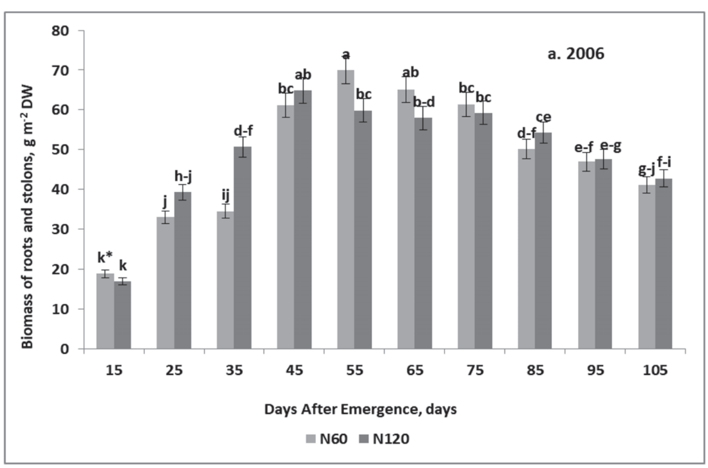


Figure 1. *Cont.*

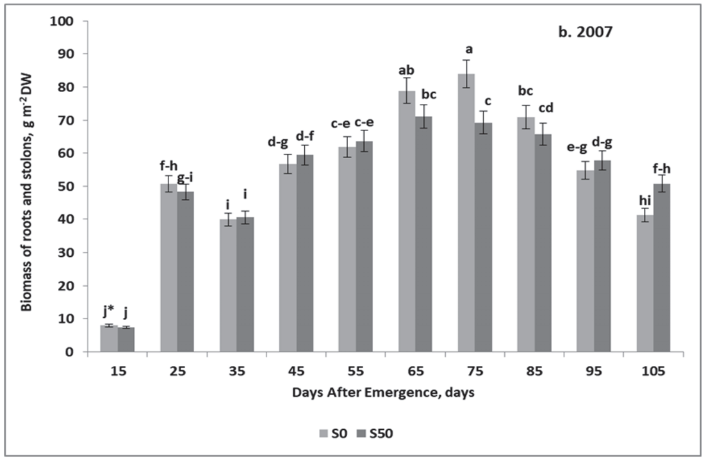
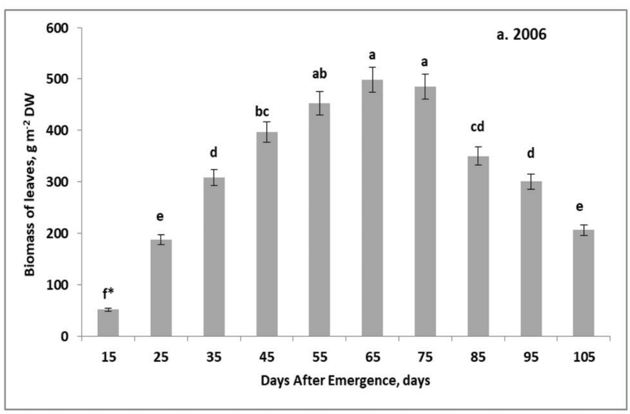


Figure 1. Seasonal trends of roots and stolons biomass growth. (**a**) 2006; (**b**) 2007. * HSD—calculated separately for each year; letters indicate significant differences between treatments ($p < 0.05$). Legend: S0, S50—sulfur rates of 0 and 50 kg ha^{-1}. Vertical bars represent standard error.



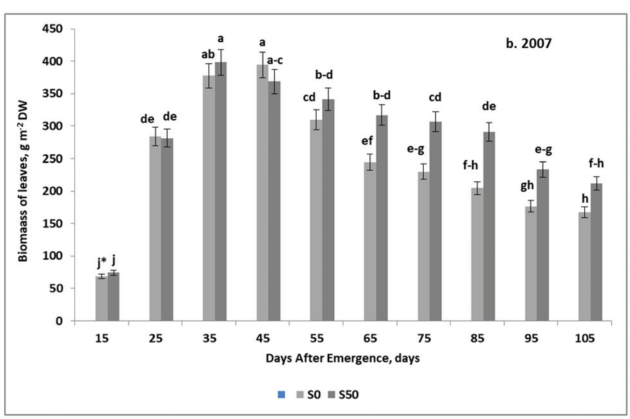


Figure 2. Seasonal trends of leaves biomass growth. (**a**) 2006; (**b**) 2007. * HSD—calculated separately for each year; letters indicate significant differences between treatments ($p < 0.05$). Legend: S0, S50—sulfur rates of 0 and 50 kg ha^{-1}. Vertical bars represent standard error.

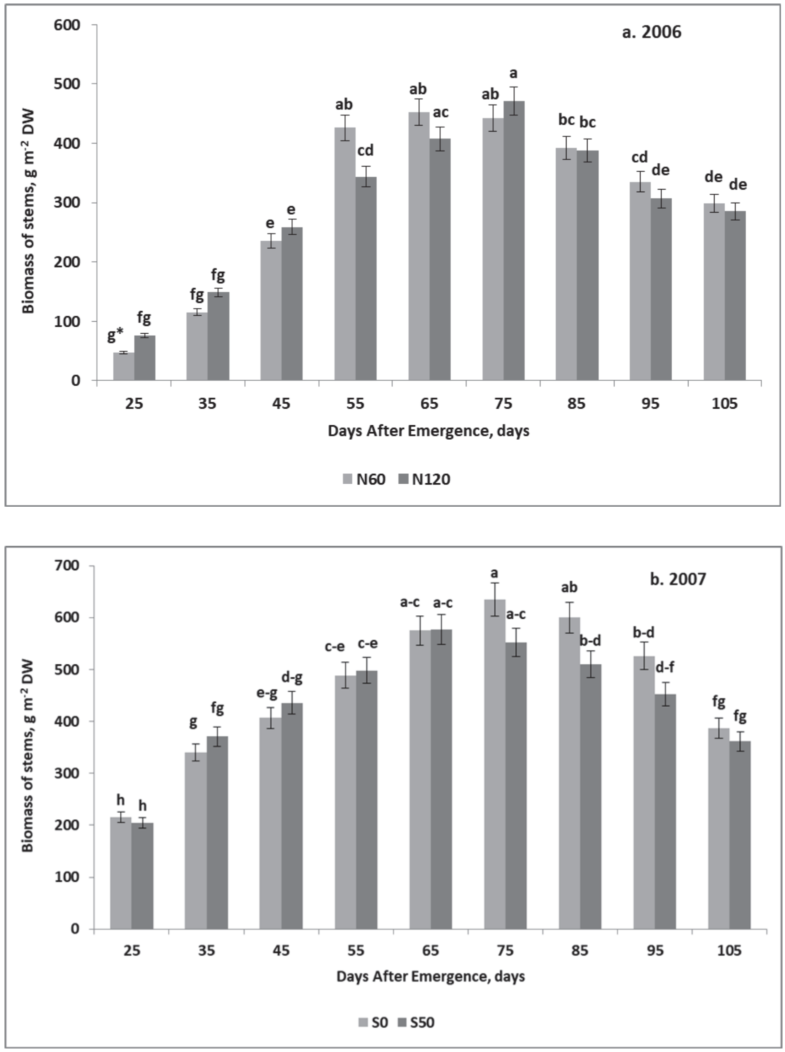


Figure 3. Seasonal trends of stems biomass growth. (**a**) 2006; (**b**) 2007. * HSD—calculated separately for each year; letters indicate significant differences between treatments ($p < 0.05$). Legend: S0, S50—sulfur rates of 0 and 50 kg ha^{-1}. Vertical bars represent standard error.

In the 2007 growing season, the increase in TTB depended on the effect of the S × T interaction (Table 1, Figure 4b). A significant and simultaneously progressive increase in TTB was recorded from the 35th DAE. But the variation due to S treatments was revealed from DAE 85. The effect of S assessed only against the background of N fertilization, i.e., for the S0 plot, increased from 56 g m^{-2} DW in the 35th DAE to 372 g m^{-2} DW at harvest. The observed differences resulted from a significantly different rate of TTB increase in the growing season:

$$\text{S0: TTB} = 18.65\ \text{DAE} - 618.9, \text{ for } R^2 = 0.98,\ n = 8, \text{ and } p \leq 0.01 \quad (2)$$

$$\text{S50: TTB} = 22.84\text{DAE} - 703, \text{ for } R^2 = 0.99,\ n = 8, \text{ and } p \leq 0.01 \quad (3)$$

The growth rate of TTB in the S0 treatment was 18% lower compared to that with added S. The value of the direction coefficient (CD) of the equation developed for the S50 was almost the same as in 2006.

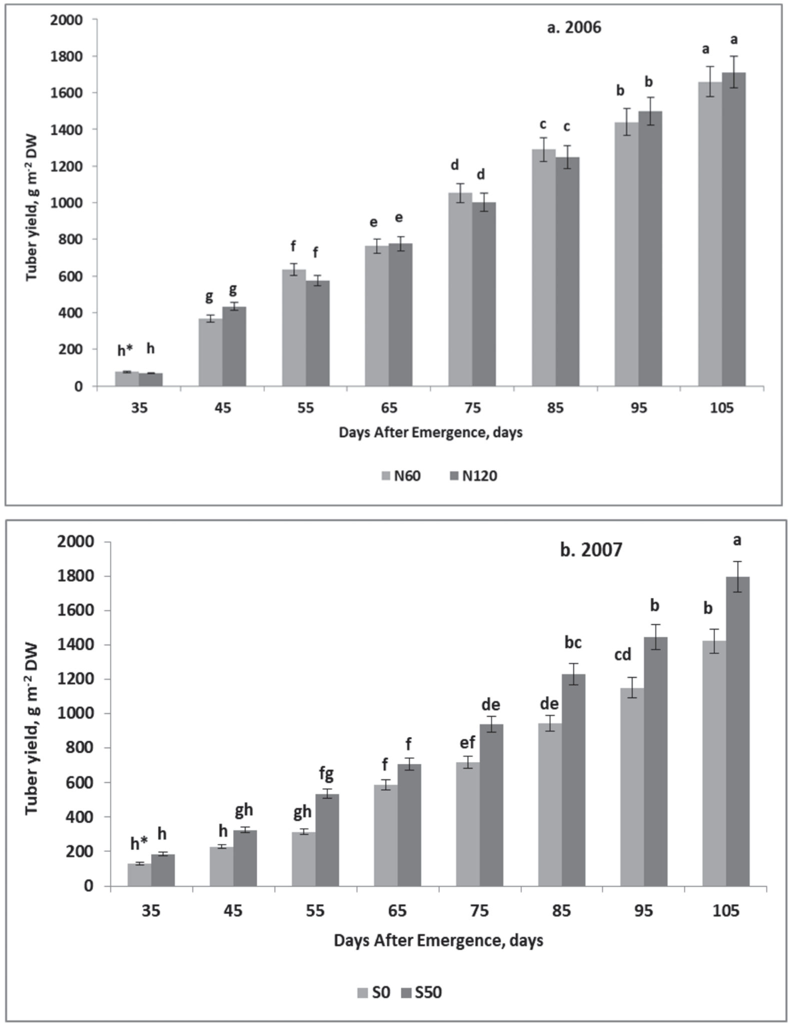


Figure 4. In-season development of the temporary tuber biomass. (**a**) 2006; (**b**) 2007. * HSD—calculated separately for each year; letters indicate significant differences between treatments ($p < 0.05$). Legend: N60, N120—nitrogen rates of 60 and 120 kg ha^{-1}; S0, S50—sulfur rates of 0 and 50 kg ha^{-1}. Vertical bars represent standard error.

2.2. In-Season Trends in Potato Non-Storage Organs Development

Seasonal trends in the growth of the non-storage potato organs are presented only for treatments decisive for the temporary tuber yield. In 2006, the whole period of roots + stolons biomass (RS) growth can be divided into three sub-phases (Figure 1a):

(1) Progressive, which lasted until the 45th DAE;
(2) Stagnation, which lasted from the 45th to the 75th DAE;
(3) Depressive, which started from the 75th DAE and lasted until the potato harvest.

The 2006 growth trend of RS was best described by the quadratic regression model (see Figure A1a for details). The maximum RS of 62.5 g m^{-2} was recorded on the 66th DAE. In 2007, the seasonal growth course of these organs changed significantly on the 35th DAE (Figure 1b). In this particular phase, there was a significant decrease in RS biomass compared to its value observed on the 25th DAE. In subsequent stages, the increase in RS was consistent with the quadratic regression model. The regression models presented below do not take into account the data from the 25 DAE:

$$\text{S0: B-RS} = -0.024\text{DAE}^2 + 3.35\text{DAE} - 41.9 \text{ for } n\ 9, R^2 = 0.93, p \leq 0.01 \quad (4)$$

$$\text{S50: B-RS} = -0.019\text{DAE}^2 + 2.79\text{DAE} - 30.4 \text{ for } n\ 9, R^2 = 0.99, p \leq 0.01 \quad (5)$$

In the ascending phase of RS growth, its main characteristic feature was a much steeper increase in biomass in the S0 variant in relation to the S50 variant. A reverse trend was observed in the descending phase of RS growth. In the case of the S0, the RS maximum of 75 g m^{-2} DW was reached on the 70th DAE. For the S50, the net increase in RS of 72 g m^{-2} was recorded on the 73rd DAE.

Seasonal growth trends of potato leaf biomass (LE) were completely different in the studied years. In 2006, the increase in LE during the growing season was best reflected by the quadratic regression model (Figure 2a). The progressive increase in LE continued up to the 65th DAE, reaching a maximum of 470 g m^{-2} (see Figure A1a for details). In 2007, the seasonal course of LE was best represented by the double-linear model (Figure 2b, and see Figure A1b,c for details). The most important attributes of the models obtained, regardless of the fertilization treatments, are:

(1) Steep initial rise, peaking just after the 35th DAE;
(2) A slight decline, after reaching the maximum biomass;
(3) Significantly less decline on the S50 variant compared to the S0.

The ascending sub-phase of this model was short and steep. The maximum LE for both treatments was recorded on the 30th and 34th DAE and amounted to 407.7 and 393.3 g m^{-2} DW for the S0 and the S50, respectively. In contrast, the descending sub-phase of LE growth was long and smooth. A slightly slower rate in LE decline was recorded for the S50 in relation to the S0. During this period, a distinct and much higher biomass was observed in the S50. The observed difference was most pronounced between the 65th DAE and the 85th DAE.

Seasonal growth trends of stems biomass (ST), regardless of the year and applied fertilizers, were consistent with the quadratic regression model. In 2006, a maximum ST of 425.2 g m^{-2} DW was recorded on the 75th DAE (Figure 3a). In 2007, the course of the ST was significantly influenced by fertilization. The differences between the sulfur treatments up to the 75th DAE were slight or negligible (Figure 3b). Significant differences resulting from S application were noted during in the descending phase of the stem biomass growth. These differences are reflected in the biomass maxima, and respective DAE optima. In the case of S0, the ST maximum of 590.4 g m^{-2} DW was reached in the 74th DAE. The corresponding values for the S50 were 555.1 g m^{-2} DW and DAE 76.

2.3. Competition Analysis of Tubers and Non-Storage Potato Organs Growth

The seasonal pattern of TTB growth, in spite of being significantly influenced by weather, was consistent with the linear regression model (Figure 4).

The source–sink relationship between enlarging tubers and competitive organs was analyzed in two distinct ways. The first criterion of the studied competition between potato parts focused on the determination of the critical day of potato vegetation i.e., the DAE_{crit} value. DAE_{crit} defines the potato vegetation period, in which the tuber biomass exceeded that of the competing part of the potato. The presented results refer to treatments, which were found to be decisive for the yields of tubers (Figure 4).

Diagrams of the growth of particular potato organs treated as a function of time (T, DAE) are presented in Figure A1a–c. The models obtained in 2006, as shown earlier in Section 2.2, are best described by the quadratic regression model. DAE_{crit} was the longest for LE, 10 days shorter for stems and 14 days shorter for roots and stolons (Table 2). DAE_{op}, indicating a period of net growth of potato organs competing with tubers, appeared for all potato organs at almost the same time, i.e., around the 66th DAE. The values of the next index, i.e., DAE_{diff} were the longest for RS and the shortest for LE. In 2007, the DAE_{crit} indices showed clearly different trends compared to 2006. First, the DAE_{crit} values for LE appeared much earlier than in 2006. Secondly, they appeared, regardless of S fertilization, after the leaves reached the maximum biomass, i.e., DAE_{op}. As a consequence, DAE_{diff} showed a negative value, especially in the S0 treatment, which preceded the DAE_{op} by 10 days. In the case of the S50, the DAE_{diff} was also negative, but it was approaching zero. It should be emphasized that both indices, i.e., DAE_{op}, and especially DAE_{crit} for S $\times$ T interaction, appeared much later than those observed in 2006. The DAE_{crit} for the S0 was longer by three, and for the S50 by two weeks. In the case of DAE_{op}, these differences were 7.3 and 4.1 days, respectively.

Table 2. Indices describing competition between growth of tubers and non-storage organs.

Plant Part	DAE_{op}	DAE_{crit}	DAE_{diff}	B_{max}, g m^{-2} DW
		2006		
Roots + stolons, RS	66.1	33.0	33.1	63.4
Leaves, LE	64.7	47.2	17.5	470.4
Stems, ST	66.4	37.4	29.0	422.2
		2007, S0		
Roots + stolons, RS	67.9	31.8	36.1	74.5
Leaves, LE	41.7	51.9	−10.2	403.3
Stems, ST	73.7	58.6	15.1	590.4
		2007, S50		
Roots + stolons, RS	70.5	27.0	43.5	70.0
Leaves, LE	46.0	46.4	−0.4	394.5
Stems, ST	70.3	51.5	18.8	551.2

Legend: DAE_{op}—the optimum day for the maximum biomass of potato organs (B_{max}) competing with tubers; DAE_{crit}—DAE at which biomass of tubers exceeded biomass of a particular non-storage potato organ; $DAE_{diff} = DAE_{op} - DAE_{crit}$.

The second criterion for assessing internal competition between the growing organs of the potato was the analysis of the source–sink relationship. In the performed allometric analysis, the potato tuber was treated as a pure sink of assimilates, and the potato's non-storage organs as their source for the growing tubers. The basic parameters of the solved regression models were (i) optimal, i.e., temporary tuber biomass (TTB_{crit}), and (ii) the corresponding, maximum biomass of the competing potato part (B_{max}). The intersection of these two regression equations determined the TTB_{crit} value. The slope of the obtained linear models was used as the competition index (CI) between the tuber growth rate and the growth rate of the remaining potato organs (Table 3). In 2006, the values of the CI indices for potato organs competing with tubers were as follows:

1. Ascending phase: 0.04 (RS) < 0.27 (LE) < 0.45 (ST, g g^{-1} tuber DW);
2. Descending phase: −0.41 (LE) < −0.26 (ST) < −0.03 (RS g g^{-1} tuber DW).

An analysis of the 2006 dataset clearly highlights that the stems and leaves were the most unstable part of the potato in response to the seasonal dynamics of tuber growth (Figure 5a). The stems showed the highest growth dynamics, i.e., a considerable increase in their own biomass per unit of tuber biomass in the ascending phase, and a mild decrease in the descending phase. The reverse pattern was noted for leaves.

Table 3. Indices describing competition between growth of tubers and competitive organs.

Ascending Phase	Descending Phase	TTB_{crit}, g m^{-2} DW	B_{max}, g m^{-2} DW
	2006		
B-RS = 0.044 TUY + 41.1 $n = 3$, $R^2 = 0.90$, $p \leq 0.05$	B-RS = −0.03 TUY + 88.1 $n = 4$, $R^2 = 0.99$, $p \leq 0.01$	652.8	69.8
B-LE = 0.27 TUY + 288.1 $n = 4$, $R^2 = 0.99$, $p \leq 0.01$	B-LE = −0.041 TUY + 894 $n = 4$, $R^2 = 0.98$, $p \leq 0.01$	888.4	529.7
B-ST = 0.45 TUY + 91.9 $n = 4$, $R^2 = 0.90$, $p \leq 0.05$	B-ST = −0.26 TUY + 716.4 $n = 4$, $R^2 = 0.97$, $p \leq 0.01$	883.3	486.7
	2007, S0		
B-RS = 0.067 TUY + 36.7 $n = 5$, $R^2 = 0.94$, $p \leq 0.01$	B-RS = −0.06 TUY + 127.6 $n = 3$, $R^2 = 0.99$, $p \leq 0.01$	693.9	85.3
Stagnation, (DAE 35 + 45)	B-LE = −0.13 TUY + 362.3 $n = 6$, $R^2 = 0.96$, $p \leq 0.01$	-	383.2
B-ST = 0.48 TUY + 300.7 $n = 5$, $R^2 = 0.96$, $p \leq 0.01$	B-ST = −0.45 TUY + 1029.2 $n = 3$, $R^2 = 0.99$, $p \leq 0.01$	788.4	676.0
	207, S50		
B-RS = 0.06 TUY + 29.9 $n = 4$, $R^2 = 0.86$, $p \leq 0.05$	B-RS = −0.023 TUY + 91.4 $n = 4$, $R^2 = 0.97$, $p \leq 0.01$	741.0	74.4
Stagnation, (DAE 35 + 45)	B-LE = −0.13 TUY + 400.6 $n = 8$, $R^2 = 0.94$, $p \leq 0.01$	-	383.2
B-ST = 0.38 TUY + 303.4 $n = 4$, $R^2 = 0.99$, $p \leq 0.01$	B-ST = −0.23 TUY + 776.4 $n = 4$, $R^2 = 0.98$, $p \leq 0.01$	778.0	599.8

Legend: B—biomass, RS—roots and stolons, LE—leaves, ST—stems, TU—tubers, TUY—tuber yield; TTB_{crit}—critical temporary tuber biomass, B_{max}—maximum biomass of the competing potato part.

For 2007, the CI indices are presented for the main treatments (Figure 5b,c):

1. S0:
    a. Ascending phase: 0.07 (RS) < 0.48 (ST, g g^{-1} tuber DW);
    b. Descending phase: −0.45 (ST) < −0.13 (LE) < −0.06 (RS g g^{-1} tuber DW).
2. S50:
    a. Ascending phase: 0.07 (RS) < 0.48 (ST, g g^{-1} tuber DW);
    b. Descending phase: −0.45 (ST) < −0.13 (LE) < −0.06 (RS g g^{-1} tuber DW).

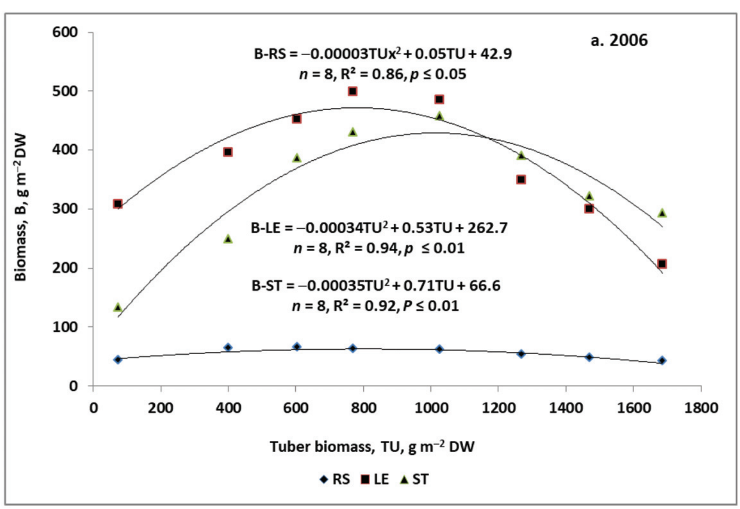


Figure 5. *Cont.*

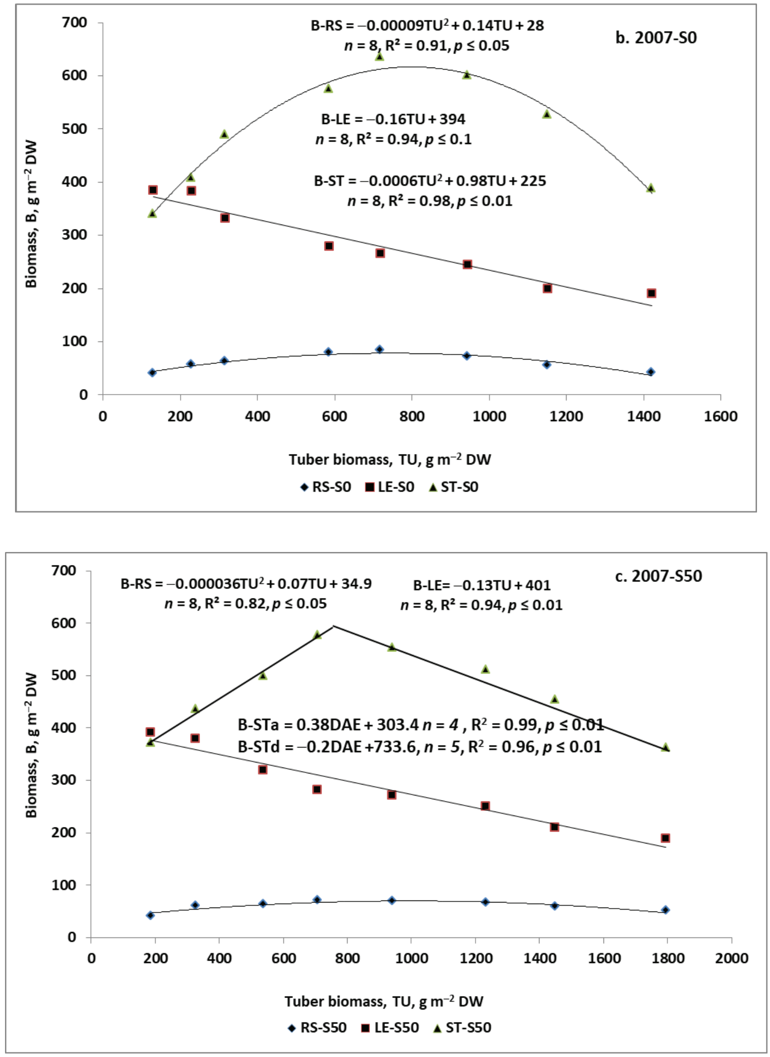


Figure 5. Sink–source relationships between the growth of tuber biomass and growth of competitive organs. (**a**) 2006; (**b**) 2007-S0; (**c**) 2007-S50. Legend: RS—roots and stolons, LE—leaves, ST—stems, TU—tubers; B-STa—ascending phase; B-STd—descending phase.

In 2007, the most distinct seasonal trends in biomass development were observed for leaves. The stagnation phase was revealed in the early stages of tuber growth. In the case of the plot fertilized only with N (the S0 plot), this phase extended from the 35th to the 55th DAE. For the S50 variant a slightly shorter lag-phase was observed. In subsequent stages of potato growth, the leaf biomass showed a mild decline, lasting until harvest (Figure 5b). In the main sulfur-fertilized plot (S50), the descending LE phase began as early as on the 45th DAE, showing a mild decline towards maturity (Figure 5c). The stems showed markedly different growth trends in response to S application. In the S0 plot, the ST growth rate in both stages was both the highest and the most long-lasting. In the main plot with N + S addition, the ST growth rate in the ascending phase was only slightly lower than in the S0 variant, but the rate of decline in the descending phase was twice as low.

2.4. The In-Season Growth and Partitioning of Total Dry Matter

The seasonal growth trends in total potato biomass were variable in response to experimental factors. In 2006, significant and, at the same time, stable differences between fertilization treatments appeared at the earliest on the 75th DAE and persisted until harvest. Considerably higher potato biomass (B) was recorded in the N60S50 and N120S0 plots (Figure S1a). The trends obtained generally best fit the quadratic regression model. However, DAE_{op} during the growing season, as recorded on the 102nd DAE, was only achieved for the N60S0 plot. In the case of other treatments, DAE_{op} appeared after the harvest. Despite the seasonal variability of B, the tuber yield was independent from the experimental factors (Figure 4a). In 2006, the increase in B during the growing season, calculated on the basis of averaged data, was consistent with the quadratic regression model (Figure S2a):

$$B = -0.245DAE^2 + 55.5DAE - 867.6 \text{ for } n = 10, R^2 = 0.98 \text{ and } p \leq 0.01 \quad (6)$$

The obtained trend clearly indicates that the theoretical increase in potato biomass in 2006 was longer than the date on which the potatoes were harvested. The optimal harvest date, as indicated by the DAE_{op}, would be reached on the 113th DAE.

In 2007, the increase in B during the growing season was variable, but significantly driven by the S × T interaction (Figures S1b,c and S2b,c). Generally, the linear trend best describes the in-season increase in Bs:

$$\text{S0: } B = 20.8 \text{ DAE} + 37.4 \text{ for } n = 19, R^2 = 0.95, p \leq 0.01 \quad (7)$$

$$\text{S50: } B = 24.1 \text{ DAE} + 15.1 \text{ for } n = 19, R^2 = 0.97, p \leq 0.01 \quad (8)$$

The obtained models clearly show that the in-season growth of B on the sulfur-fertilized plot was 16% faster than on plots with N only. The differences, first observed on the 25th DAE, persisted until harvest. Significant differences between the two treatments did not become significant until the 55th DAE. Greater B in the plots treated with S was recorded in the final stages of potato yield development. As a result, the yield of tubers at harvest was 26% higher on the plots fertilized with sulfur compared to those fertilized only with N.

In both study years, regardless of the course of the weather, the value of the potato harvest index PHI increased progressively in the growing season (Table 1). The in-season trend of PHI, regardless of the year and fertilization treatments, was consistent with the linear regression model and was significant only for the observation (T):

$$\text{2006: } PHI = 0.8 \text{ DAE} - 7.28 \text{ for } n = 8, R^2 = 0.95, p \leq 0.01 \quad (9)$$

$$\text{2007: } PHI = 0.8 \text{ DAE} - 12.6 \text{ for } n = 8, R^2 = 0.99, p \leq 0.01 \quad (10)$$

The main difference between the two equations is the constant, which was much lower in 2007. The difference between the years began directly at DAE 35, reaching the highest values in the tuber bulking phase (from 45 to 55 DAE). In 2006, PHI in this particular period was more than 10% higher than in 2007. Final PHI in 2006 reached 76% and in 2007 it was 72% (Figure S3).

The seasonal structure of the dry matter partition between potato organs is shown in Figure S3. For each part of the potato competing with tubers, a seasonal downward trend in the share of biomass during the growing season was observed. The relative share of roots + stolons in the B decreased according to the power function in 2006, and linearly in 2007. The seasonal trend in leaf biomass in the B, regardless of the year and fertilization treatments, corresponded best to the power function. Much more diverse patterns were observed for the stem biomass, which was best described by the quadratic regression model (Figure 6). The differences between the years refer to:

(1) Share of the stem biomass in the B in the early stages of tuber growth;
(2) The length of the stability period of stem biomass share.

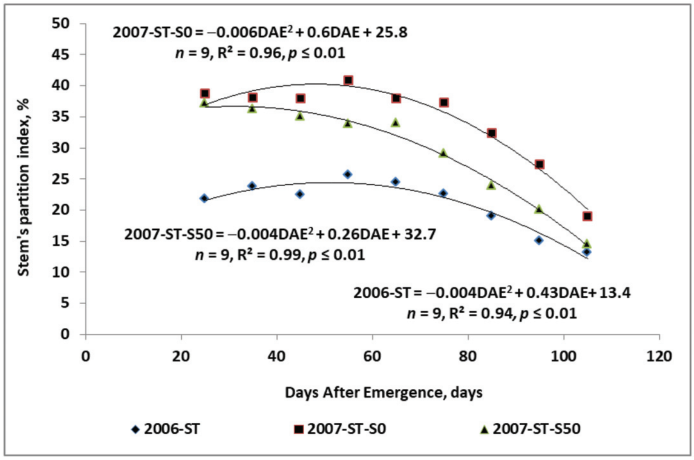


Figure 6. Seasonal trend in the relative share of stem biomass in the total potato biomass. Legend: N60, N120—nitrogen rates of 60 and 120 kg ha^{-1}; S0, S50—sulfur rates of 0 and 50 kg ha^{-1}.

In 2006, the percentage of stems in total potato biomass ranged from 22% on DAE 25 to a maximum of 26% on DAE 55. At harvest, it declined to 13%. In 2007, the initial share of stems in DAE 25 was 38%. The application of sulfur significantly influenced the general trend of stems contribution in the total potato biomass. Their share on the S0 plot showed a significant increase, reaching 41% on the 55th DAE, while on the S50 plot it decreased smoothly from DAE 35. The stabilization phase, assuming a 4% difference between consecutive observations was 50 days in 2006, while in 2007 it reached 60 days for the S0, and 50 days for the S50.

3. Discussion

3.1. Tuber Yields and Seasonal Trends of Potato Tubers Growth

The in-season development of the potato tuber yield is a function of the weather conditions during the growing season and the supply of nutrients [12,14]. Our study confirmed this opinion, but only partially. In this particular case, the final yield of tubers, however, was not dependent on the course of the weather, even though the weather conditions were completely different. Under climatological conditions in Poland, the highest tuber yield can be obtained provided there is medium precipitation in May (45 mm) and June (65 mm), and high precipitation in July (90 mm) and August (75 mm) [23]. The environmental conditions, evaluated based on the Sielianinov hydrothermal indices, indicated a severe drought in 2006 (0.58), and optimal weather conditions in 2007 (1.34) [24]. The final tuber biomass averaged for experimental treatments, was 1687 g m^{-2} DW in 2006 and 1608 g m^{-2} DW in 2007. The biomass of tubers recalculated into fresh weight was 84.3 t ha^{-1} (20% DM content) in 2006, and 86.6 t ha^{-1} (18.5% DM content) in 2007. Tuber yields at this level under natural precipitation are possible, but only if the soil is very fertile [25]. In the dry 2006, regardless of the N rate, which was 60 and 120 kg ha^{-1}, the tuber yield was at the same level. The lack of response by the potatoes to N doses indicates a high mineralization potential of the soil to supply the plants with N in the phases of intensive tuber growth [3,26]. A positive impact of S^0 on tuber biomass was found each year, but it was significant only in the optimal 2007 (Figure 4b). At harvest, the average biomass of potato tubers on the main plot fertilized only with N was lower by 21% than that receiving sulfur at the rate of 50 kg ha^{-1}. The significant increase in the tuber yield due to S fertilizer can be partly explained by the content of its available form in the soil prior to planting the potatoes. The data obtained support the results of other studies, but the effect of S in our study was more striking [27,28]. On the basis of the tuber yield, it can be stated that S is an effective yield-forming factor for potatoes.

The seasonal pattern of potato tuber yield growth, confirming the physiological maturity of tubers, should meet the conditions of the quadratic or any sigmoidal regression model [14]. In our study, the seasonal trends in tuber biomass growth were only seemingly consistent with the quadratic regression model in 2006, and the linear regression model in 2007. In 2006, the DAE_{op} for B appeared shortly after the harvest date, indirectly indicating the slightly immature status of harvested tubers. The linear model obtained in 2007 is a direct indicator of the immature physiological status of potato plants at harvest [29].

The general, linear trend of tuber yield increase during the growing season is consistent with the theory, indicating a dependence of the crop yield on the source–sink relationship during the growing season [30,31]. On the basis of the linear growth pattern of potato biomass, it can be concluded that under favorable environmental and soil conditions for the potato, tuber yield is determined by the sink strength. This means that tuber activity in the studied case was the yield driver that affected the source activity. This type of seasonal tuber yield growth suggests, however, that the yielding potential of the tubers, i.e., their sink capacity for assimilates, was not realized in the studied case [14].

3.2. Growth Competition between the Tuber and Non-Storage Potato Organs

In the classical approach to the source–sink relationship, the strength of the physiological sink depends on the source activity [2]. This hypothesis was verified during this study. The yield of potato tubers can be evaluated as a result of the relationships between the seasonal growth rate of tubers and the growth rate of other potato organs, which compete with tubers for assimilates. The set of competitive potato organs includes leaves, steams, stolons, and true roots [7]. The obtained linear model of tuber growth during the growing season, regardless of weather conditions, clearly indicates that the enlarging tubers act as a pure physiological sink (Figure 4).

The source–sink relationships in potatoes require recognition of the state of its organs' biomass, in response to tuber initiation. As it results from the analysis of the dynamics of the growth of potato organs, the impact of tuber initiation on the source growth depended on the course of the weather during the pre-tuberization stages of potato growth. The growth habit of vines in response to weather in both years was different. The pattern of leaf growth was governed up to early tuber bulking by temperature. In 2006, leaves stopped growing on the 66th DAE, whereas in 2007, it was 18 days earlier (48th DAE). The main reason for the observed differences was the temperatures in July. In 2006, air temperature exceeded 25 °C, but in 2007, it was below 20 °C. Under relatively low temperature the numbers of branches and leaves associated with them are lower. The change point is 25 °C, which results in the appearance of long stems, but with smaller leaves [32]. The tuber sink strength, defined by its initial weight at the beginning of the bulking phase, determines its requirements for assimilates in the subsequent stages of growth [13]. In potatoes, leaves are a pure physiological source of assimilates, which also act as a temporary sink, using a part of the fixed CO_2 to build their own biomass [7,33]. Other vegetative organs that compete with the enlarging tubers can also be regarded as temporary sinks. The critical value of the temporary tuber biomass (TTB_{crit}) was used as a criterion for evaluating the in-season competition between enlarging tubers and the growth rate of other potato organs. TTB_{crit} defines the critical growth rate of the TTB required to break down the dominance of the potato organs that compete with tubers for assimilates. The TTB_{crit} cannot be treated as a single point. It can be considered as a period of a different length, in which the growth rate of tubers and the competitive organ are temporarily balanced. Based on TTB_{crit}, the entire growth period of potato vegetative parts competing with the growing tubers can be divided into three sub-phases:

(1) Ascending, having the status of a temporary sink;
(2) Stagnating, which is a transition stage between the sink and a pure source phase;
(3) Descending, having the status of a pure source.

The property of the first period is a net gain in the biomass of all potato organs. This state indicates a lack of competition between the potato organs for assimilates. The descending growth phase of potato organs competing with tubers is the period of pure remobilization of dry matter from each potato organ into enlarging tubers.

In both years, the growth of potato organs competitive with tubers did not change the linear pattern of tuber yield increments during the growing season (Figure 4). The tuber sink strength can therefore be considered as the factor forcing both the size of the pure source, i.e., leaf biomass, and the biomass of other temporary sink organs. These facts absolutely support the hypothesis by Körner [30,31], suggesting the primacy of the net sink over the net source.

The observed relationships cannot be explained without taking into account the course of weather and experimental factors. As reported by Li et al. [3], the in-season C reallocation is to some extent, modified by environmental and agronomic factors. In our case, in the dry 2006, the relationship between the biomass of tubers and leaves during the growing season was described by the quadratic regression model. In 2007, the seasonal trend in leaf biomass showed a completely different pattern. As a rule, leaf biomass decreased from the beginning of tuber enlargement. The degree of the LE decline, supported by values of the direction coefficient, was steeper on the plot fertilized only with N. This type of LE trend can be explained by the accelerated rate of leaf dry matter remobilization due to the progressively increasing pressure of the enlarging tubers [14]. Potato plants fertilized with S showed a much slower depression of LE biomass in the descending phase of this organ development. As a consequence, the final tuber yield on plots fertilized with both N and S was 26% higher. The obtained final result of the tuber yield not only confirms, but also explains the reason for the high response of the potato to the application of sulfur [27,28].

The production functions of leaves in potatoes can be explained by analyzing the seasonal trends of LE. They are described by indicators such as DAE_{crit} and DAE_{op}. It should be strongly emphasized that the DAE_{crit} for LE in 2006 appeared long before the DAE_{op}, but in 2007 it was much later. In this particular year, the difference between both indices, expressed as DAE_{diff}, was negative. The difference obtained clearly indicates that tuber biomass exceeded that of leaf biomass before it reached its maximum. Thus, in full potato vegetation, DAE_{crit} for LE can be treated an important indicator of the final tuber yield:

$$\mathrm{TBU} = -63.8\mathrm{D}_{crit} + 4728 \text{ for } n = 3,\ \mathrm{R}^2 = 0.98, \text{ and } p \leq 0.01 \quad (11)$$

The equation obtained confirms the occurrence of competition for assimilates between leaves and progressively growing tubers. This type of relationship between the growth of leaves and tubers stresses the often-discussed fact of the excessive growth of leaves as a factor disturbing the growth of tubers [33]. In our study, the observed competition revealed itself under a certain set of environmental and agronomic conditions, i.e.:

(1) In a year of favorable growth conditions for potatoes at the beginning of vegetation;
(2) On soils with high natural fertility (high content of humus and available nutrients);
(3) Under a good supply of nitrogen, including its applied dose.

In agronomic practice, these conditions are frequently observed under irrigation [34]. Our study showed that the application of 50 kg S ha^{-1} in the form of elemental sulfur resulted in significant DAE_{crit} shortening. As a result, the final tuber yield in the N + S variant approached the maximum value in the studied case.

The missing information in tuber yield development with respect to the in-season competition between tubers and non-storage potato organs mainly refers to the production function of the stems. This potato tissue, as a temporary sink, is rich in proteins, carbohydrates, and mineral nutrients [32,35]. The growth rate of the stem biomass in the ascending phase was far below 1.0, which only seemingly stresses the lack of competition for assimilates with tubers. In both years, the stem growth rate in the ascending phase was sufficiently high, but did not exceed +0.45 g g^{-1} of tuber DW. In the descending phase, it was significantly affected by year and fertilization treatments. In 2006 for all treatments and in 2007 for treatments with S, this parameter was almost similar, varying from −0.26 to −0.23 g g^{-1} tuber DW. In 2007, this parameter on plots fertilized only with N was −0.45 g g^{-1} of tuber DW. A much lower rate of stem biomass decrease on plots fertilized with S, concomitant with a higher tuber yield, suggests that the stems act as an assimilate buffer for the growing tubers. The quadratic regression model of the relationship between tubers and stems growth, dominating in all treatments, indicates the presence of the stagnation period in the main phase of tuber bulking, which stabilized tuber growth (Figure 5). The buffer function of stems was also corroborated by their share in the total tuber biomass (Figure 6). The rate of stem growth was significantly modified by application of elemental S. The observed response of the stem biomass to environmental factors and agronomic measures corroborates the hypothesis of stem plasticity to the growing conditions [36]. This specific function of the potato stem largely explains the increase in tuber yield in response to S application [25,26].

White et al. [6] concluded that the increase in crop productivity requires a quantitative definition of the extent to which sources or sinks limit crop plant growth, and this changes during development. The quantitative relationships between the potato organs during the growing season are much more complex than is often assumed in even very sophisticated models of potato growth and yielding [3,8]. Our study provided not only a few sets of basic data on seasonal trends in the biomass of roots + stolons, leaves, stems, and finally tubers, but also showed allometric relationships between tubers and non-storage potato organs.

4. Materials and Methods

4.1. Experimental Site

A field experiment was carried out at Kicin (52°46′ N, 17°02′ E, Poland) on soil originating from a silty clay loam classified as Chernozems loamic [36]. The content of organic (C_{org}) in a 0.0–0.3 m layer was 33 ± 0.9 and 27 ± 0.1 g kg^{-1} soil (loss-on ignition); pH was 6.6 and 7.0 (1.0 M KCl) in 2006 and 2007, respectively. The content of available nutrients, measured before the application of fertilizers, was very high for P (305 ± 71 and 267 ± 28); medium and low for K (253 ± 117 and 160 ± 13); low for Mg (117 ± 14 and 106 ± 39); low for Ca (1605 ± 137 and 1678 ± 618) mg kg^{-1} soil for 2006 and 2007, respectively (Mehlich 3 method) [37,38]. The amount of the mineral N (N_{min}), measured in a 0.0–0.6 m layer, was 60 ± 6 and 83 ± 11kg ha^{-1} in 2006 and 2007, respectively [39]. The amount of available sulfur (S-SO_4) was 10.3 ± 2.4 and 7.4 ± 1.0 mg kg^{-1} dry soil, in 2006 and 2007, respectively [40].

4.2. Weather Conditions

The in-season differences in potato biomass resulted from the course of the weather in the early stages of plant growth. In both years, the average monthly temperatures were higher than the long-term averages (1965–2007; 14.6 °C in May, +0.6 °C and +2.0 °C; June, 17.7 °C, +3.3 °C and +3.7 °C, in 2006 and 2007, respectively). July 2006 (20.1 °C) was extremely hot (+5.6 °C), and in 2007 it was much cooler (−0.5 °C). The total amount of precipitation in 2006 and 2007 for these three months amounted to 108 mm and 233 mm, respectively. The growth conditions evaluated on the basis of Sielianinov hydrothermal indices were significantly worse in 2006 (0.58, severe drought), compared to 2007 (1.34, optimal) [29]. The shortage of precipitation in 2006, due to the high content of soil water and nutrient resources at the time of potato planting did not disturb the development of potato biomass. The growth conditions in 2006 were significantly improved due to extremely high precipitation in August (150 mm).

4.3. Treatments and Crop Management

The field experiment was arranged as a two-factorial split-plot design, replicated 8-folds:

1. N rate (acronym N): 60 and 120 kg N ha^{-1};
2. Sulfur: without S (S0), with sulfur (S50);

3. Periodic sampling of potato plants during the potato growing season was used as a third experimental factor [40].

Nitrogen in the form of urea was applied in accordance with the experimental design in the whole rate prior to potato planting. Phosphorus at a rate of 25.8 kg P ha^{-1} as triple superphosphate (46% P_2O_5); K at a rate of 99.6 kg K ha^{-1} as muriate of potash (KCl); and sulfur as elemental sulfur (S^0) at a rate of 0, and 50 kg ha^{-1} were applied together with N two weeks before potato planting. The total area of a single plot was 58.5 m^2 (13 × 6 m) The potato variety *Zeus* was planted on the 24 April 2006 and 19 April 2007, respectively. The preceding crop to potato was spring barley. The post-barley agronomic operations for potato planting in the spring of the following year were aimed at reducing water losses and weed growth. To achieve this goal, the soil was harrowed several times after plowing. A total of 53,000 potato tubers were planted in 0.75 m rows and at 0.25 m distance within a row. Plant protection was carried out in accordance with the code of good practice. The tubers were harvested mechanically 105 days after emergence from an area of 19.5 m^2 (13 × 1.5 m).

4.4. Plant Material Sampling and Analysis

The plant material used for dry matter determination was collected from 8 plants (1.0 × 1.5 m = 1.5 m^2) per plot. Periodic sampling was performed at 10-day intervals, starting on the 15th day after full emergence until maturity (Days After Emergence, DAE): 15, 25, 35, 45, 55, 65, 75, 85, 95, and 105. Samples were taken from the soil to a depth of 30 cm from the top of the ridge. The sampled material was then divided, depending on the potato stage of growth, into subsamples of leaves (LE), above-ground stems (ST), stolon + roots (RS), and tubers (TU). The results are expressed on a dry weight basis.

4.5. Calculated Parameters

4.5.1. Growth Pattern of Potato Organs

The general pattern of the in-season potato biomass development (RS, LE, ST, total biomass—B, temporary tuber biomass—TTB) was obtained after fitting the actual results to polynomial regression models:

$$\text{Linear}: \ \text{TTB} = \text{aDAE} + \text{B} \tag{12}$$

$$\text{Quadratic}: \ \text{TTB} = \text{aDAE}^2 + \text{bDAE} + \text{C} \tag{13}$$

The main estimated parameters of this function are:

$$\text{DAE}_{\text{op}} = -\frac{\text{b}}{2\text{a}} \tag{14}$$

$$\text{TTB}_{\text{max}} = \text{c} - \frac{\text{b}^2}{4\text{a}} \tag{15}$$

where: TTB—temporary tuber biomass, g m^{-2} DW; B_{max}—maximum biomass of a particular potato organ, g m^{-2} DW; DAE—Days After Emergence; DAE_{op}—the optimum DAE; a, b, c, d—regression constants.

4.5.2. Critical Day of Tubers Growth—DAE_{critt}

Critical day of tuber growth was calculated by solving three pairs of corresponding equations, describing the seasonal growth trends of the biomass of potato organs:

TU vs. RS, b. TU vs. LE, c. TU vs. ST.

4.5.3. Competition Indices

Competition indices were calculated on the basis of the appropriate pair of linear equations, describing the ascending and descending trend in biomass growth of potato organs competing with tubers. Based on the obtained linear models, the following set of parameters was calculated for each potato organ:

1. CI index—the competition index, i.e., the growth rate of a specific potato organ per unit of tuber biomass. This parameter is equal to the value of the slope of the obtained linear equation (CD), g g^{-1} tuber;
2. TTB_{opt}—optimal value of the temporary tuber biomass for the maximum biomass of the competing potato organ, g m^{-2} DW;
3. B_{max}—maximum biomass of the potato organ competing with the tubers, g m^{-2} DW.

The general concept of the analysis of competitive relations was divided into two steps:

a. Ascending sub-phase: $B_{as} = a_{as}TU + b_{as}$;
b. Descending sub-phase: $B_d = -a_{ds}TU + b_{ds}$.

4.6. Data Analysis

The collected data were subjected to an analysis of variance using STATISTICA® 13 (StatSoft, Inc., Krakow, Poland 2013). Experimental factors, i.e., N and S rates, including observations (T) were treated as fixed effects [41]. The influence of the year was analyzed independently in order to assess the seasonal variability of examined potato traits on the experimental factors. The distribution of the data (normality) was checked using the Shapiro–Wilk test. The homogeneity of variance was checked by the Bartlett test. Means were separated by honest significant difference (HSD) using Tukey's method, when the F-test indicated significant factorial effects at the level of $p < 0.05$. Polynomial effects were used to determine (i) linear and/or quadratic growth trends of potato organ biomass, (ii) growth rates of potato organs during the season.

5. Conclusions

The potato growth pattern coded at the onset of tuberization was a decisive factor for the dry matter partitioning between the potato organs during the subsequent tuber growth phase. The tuber sink strength, expressed as a linear increment of tuber biomass in the growing season, was a key driver influencing the seasonal growth patterns of potato organs, such as leaves, stems, and roots and stolons, which competed with tubers for assimilates. Two indicators have been developed to explain the relationship between growing tubers and potato organs competing for assimilates during the growing season. The first, DAE_{crit}, defined as DAE, at which the tuber biomass exceeds that of the competing potato organ. It can be concluded that under particular growing conditions, when DAE_{crit} precedes the maximum leaf biomass, the tuber yield decreases. This phenomenon occurred when the potatoes were fertilized only with N. Sulfur fertilization was a remedy for the negative effects of nitrogen applied alone. The second index, the competition index (CI), has proven to be a reliable indicator of the seasonal evaluation of the tuber growth rate and competitive potato organs. Its prognostics value was especially revealed in the descending phase of stem growth. It can be concluded that the absolute rate of stem biomass decrease above $\frac{1}{4}$ of the tuber growth rate reduces the tuber yield.

The tuber yield-forming effect of added sulfur results from a balanced growth of stems during the ascending and the descending phase. The stabilizing action of sulfur resulted in a smooth change of the stem biomass during the descending phase. As a consequence, there was a significant reduction in the value of the CI compared to the effect of nitrogen applied alone (-0.23 g g^{-1} DW vs. -0.45 g g^{-1} DW for the S50 to S0 plots, respectively). Lower values of this index, both in the ascending and descending phase of the seasonal growth rate of stem biomass, resulted in a higher yield of tubers. Potato stems can be, therefore, treated as a tuber yield stabilizer, buffering the transfer of assimilates from leaves to the enlarging tubers.

This study clearly showed that allometric relationships between tubers and non-storage potato organs growth during the potato growing season are key factors determining the final tuber yield. The use of elemental S significantly modifies the yield-forming effect of nitrogen, leading to a higher yield of tubers.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants11030248/s1, Trends in the biomass of potato organs increase during the growing season, Figure S1. Seasonal trends of potato biomass growth. (a) 2006; (b) 2007, Figure S2. Trends in the potato biomass increase and partitioning between organs, Figure S3. Seasonal variability in the relative share of potato organs in the total biomass.

Author Contributions: Conceptualization, K.F. and W.G.; methodology, K.F. and W.G.; software, T.S.; validation, K.F., W.G., and K.P.-C.; formal analysis, T.S.; investigation, K.F.; resources, K.F. and K.P.-C.; data curation, K.F.; writing—original draft preparation, K.F.; writing—review and editing, W.G.; visualization, T.S.; supervision, W.G.; project administration, K.P.-C. All authors have read and agreed to the published version of the manuscript.

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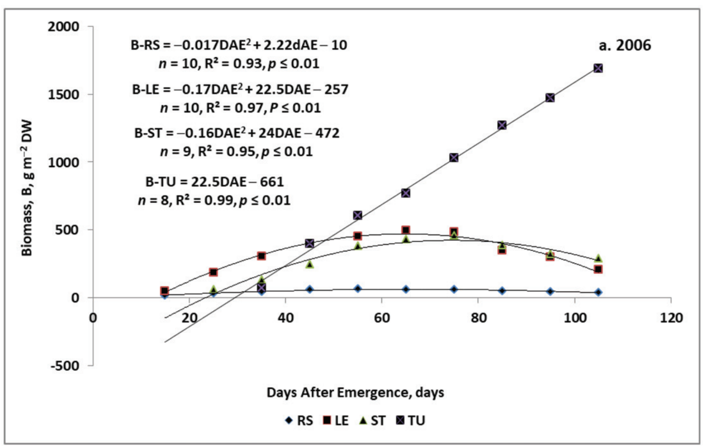
Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the private property of Karolina Frąckowiak.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A



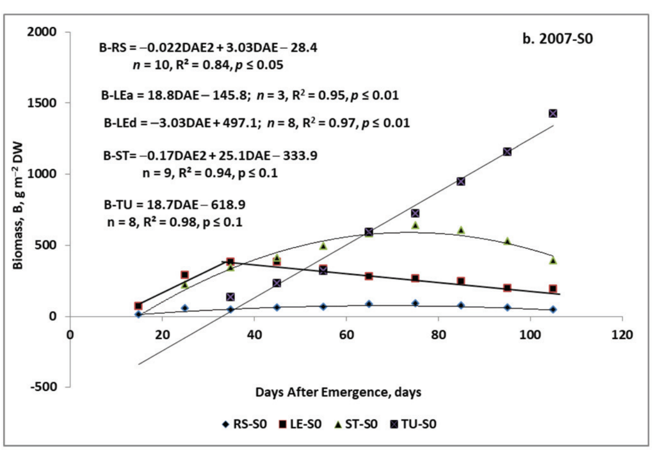


Figure A1. *Cont.*

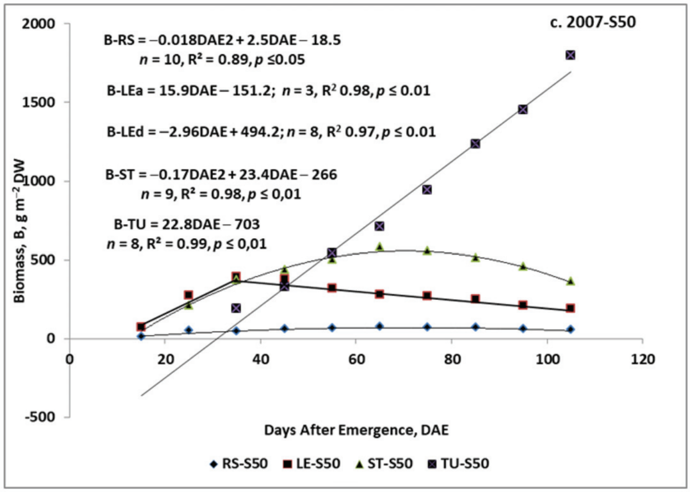


Figure A1. Trends in the biomass of potato organs increase during the growing season. (**a**) 2006; (**b**) 2007-S0; (**c**) 2007-S50. Legend: RS—roots and stolons, LE—leaves, ST—stems, TU—tubers; S0, 50—sulfur rates of 0, 50 kg ha^{-1}; B-STa—ascending phase; B-STd—descending phase.

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Reduction in Nitrogen Rate and Improvement of Nitrogen Use Efficiency without Loss of Peanut Yield by Regional Mean Optimal Rate of Chemical Fertilizer Based on a Multi-Site Field Experiment in the North China Plain

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Abstract: It is important to quantify nutrient requirements and optimize fertilization to improve peanut yield and fertilizer use efficiency. In this study, a multi-site field trial was conducted from 2020 to 2021 in the North China Plain to estimate nitrogen (N), phosphorus (P), and potassium (K) uptake and requirements of peanuts, and to evaluate the effects of fertilization recommendations from the regional mean optimal rate (RMOR) on dry matter, pod yield, nutrient uptake, and fertilizer use efficiency. Results show that compared with farmer practice fertilization (FP), optimal fertilization (OPT) based on the RMOR increased peanut dry matter by 6.6% and pod yield by 10.9%. The average uptake rates of N, P, and K were 214.3, 23.3, and 78.4 kg/ha, respectively, with 76.0% N harvest index, 59.8% P harvest index, and 41.4% K harvest index. The OPT treatment increased N, P, and K uptake by 19.3%, 7.3%, and 11.0% compared with FP, respectively. However, the average of yield, nutrition uptake, and harvest indexes of N, P, and K were not significantly affected by fertilization. The peanut required 42.0 kg N, 4.6 kg P, and 15.3 kg K to produce 1000 kg of pods. The OPT treatment significantly improved the N partial factor productivity and N uptake efficiency but decreased the K partial factor productivity and K uptake efficiency. The present study demonstrates that fertilizer recommendations from RMOR improve N use efficiency, and reduce N and P fertilizer application without yield loss in regions with smallholder farmers, and the corresponding estimation of nutrient requirements helps to make peanut fertilization recommendations.

Keywords: *Arachis hypogaea* L.; fertilizer use efficiency; nutrient uptake; optimal fertilization; pod yield

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1. Introduction

Peanut is an important oilseed crop, an N_2-fixing legume plant with drought tolerance, and a very efficient cash crop with relatively low production input, high yield, and higher price and greater income than other oil crops [1]. The extraction rate of peanut oil is high, and the oil quality is good. Therefore, peanut plantations play an important role in meeting edible oil demand and supporting local economies. China is a major peanut producer, ranking second in planting area after India and first in total production [2]. In 2020, the planting area reached five million hectares, and the total production reached 18 million tons, accounting for more than 20% and 40%, respectively, of the global totals [2]. Nitrogen

(N), phosphorus (P), and potassium (K) are essential nutrients for crops. Application of NPK fertilizer can improve crop yield [3].

Although peanut is a legume with the ability to fix atmosphere N, its growth requires adequate N, P, and K nutrients by fertilization [3]. The N fixation by rhizobia can meet 40% to 50% of the N requirement of peanut plants [4]. However, the N fixation level by peanut rhizobia is significantly and negatively correlated with increasing N application [4]. China is the world's largest consumer of chemical fertilizer [5]. In recent years, unreasonable fertilization in pursuit of higher crop yields has become common, while yield response to fertilization has become lower. Unreasonable fertilizer application not only increases production costs and reduces FUE but also causes environmental pollution [5,6]. Therefore, it is an important challenge for sustainable peanut production to ensure high peanut yield, quality, and FUE through rational fertilization.

In recent decades, studies have attempted to develop various methods to optimize the application rates of chemical fertilizer to solve the associated economic and environmental problems caused by improper application. These techniques of fertilization recommendation were based on soil testing [7], aboveground plant analysis [8], yield–fertilizer rate response modeling [9], digital and information technology [10–12], and integrated soil-crop system management [13,14]. Most of these recommended techniques have focused on site-specific optimal nutrient rates and have required field trials, soil samples, plant collection, and laboratory analysis, factors that can be rather time-consuming, labor-intensive, and expensive. Although these methods are useful for precision fertilization, they are rather difficult to implement widely for many smallholders in developing countries including China [15]. Firstly, arable land in these countries/regions is limited, with small sizes and scattered field plots. Secondly, in the rural regions of these areas, there is a lack of testing conditions and technical guidance for agrochemical services, with insufficient testing equipment and technical personnel. Finally, these field sites are highly intensive with two or sometimes three crops planted annually in the same field, and testing is difficult to perform in time to avoid delaying planting.

In the practice of agricultural production, it is critical to establish a simple method for the overall regulation and promotion of the rational application of fertilizer on a regional scale rather than a field scale [16]. Numerous factors affect crop yields, and some are difficult to predict accurately. Therefore, even for the same field, the suitable fertilizer application rate and related parameters obtained from an experiment in one year may not be accurately applied to other years [17]. Accordingly, a very high degree of accuracy is unnecessary to calculate the appropriate fertilizer application rate for obtaining a certain yield. For agricultural production, the determination of a suitable fertilizer application range can basically meet the needs of large-scale production.

A regional mean optimal rate (RMOR) of N fertilizer application has been proposed to resolve this technology gap in Tai Lake Region, China [18]. The purpose of the resolution is to provide a baseline fertilization recommendation for a specific region by determining the RMOR of this region to obtain the maximum total production and economic benefits. The RMOR can be the average of the optimal fertilizer rate obtained from the multi-site trial, and is further used as the fertilization recommendation for the whole region [18]. However, the optimal fertilizer rate can be determined by other methods, such as nutrient requirement to produce unit yield. The method has been proven feasible for crops such as rice, wheat, and oilseed rape; the recommendations have produced increasing yields, income, and nutrient use efficiency and have reduced environmental impacts [19,20]. Adjusting the RMOR for different regions, crop types, and soil conditions can provide a more accurate estimation of the optimal rate of fertilizer applied. However, the effectiveness of this resolution in other regions, crop types (N-fixing crops such as peanut), and nutrients (P and K fertilizer) is unknown.

Here, a two-year period (2020 and 2021) of multi-site field experiments were conducted in the largest peanut-growing area of the North China Plain. The hypothesis is that the ROMR method can maintain peanut yield and FUE while reducing the chemical fertilizer at a regional scale. The overall goals of this study were to quantify (1) N, P, and K uptake and requirements; (2) effects of the RMOR on peanut yield, nutrient uptake, and utilization efficiency; and hence (3) effectiveness of this RMOR method in peanut farming at the county scale.

2. Materials and Methods

2.1. Experimental Site

The field experiments were conducted in 2020 and 2021 in several townships in Zhengyang County (N32°16′-32°47′, E114°12′-114°53′), Henan, China (Figure 1). Both the peanut planting area and production of Zhengyang county rank first among all counties in China. The area is located in the transition region from the north subtropics to the warm temperate zone, and it has a continental monsoon humid climate. The average annual temperature is 15.3 °C, and the average annual precipitation is 935 mm. The monthly temperature and rainfall are shown in Figure 2 [21].

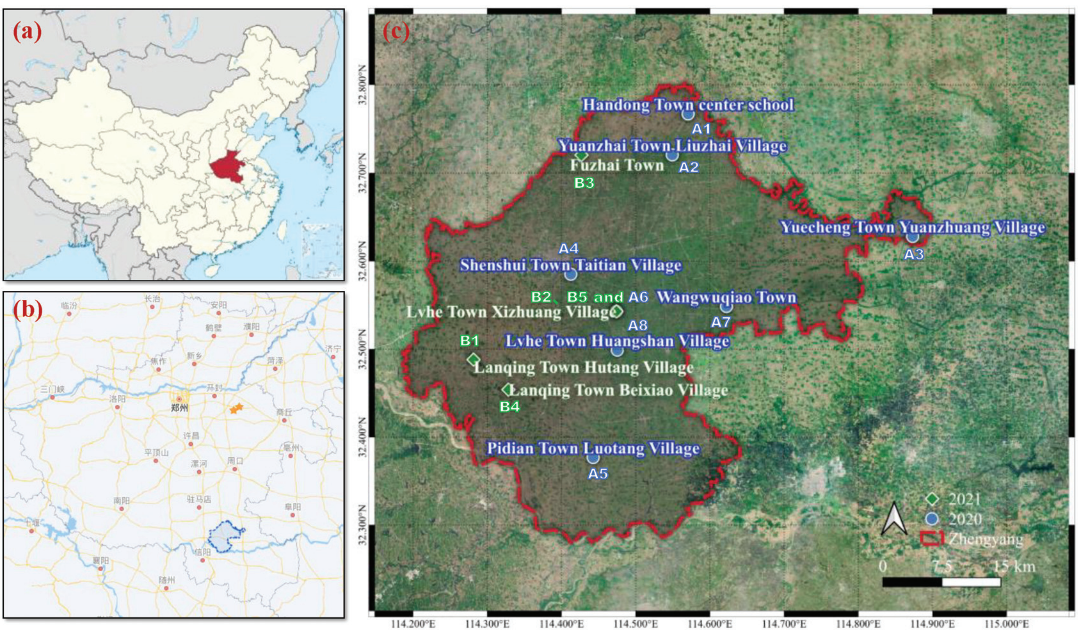

Figure 1. The geographical locations of experimental field sites. (**a**) Henan Province is shown as a red background in the China map; (**b**) the blue border is the Zhengyang County; (**c**) distribution of experimental field sites in the Zhengyang County.

2.2. Field Experiment Design

There were eight field sites in 2020 and five sites in 2021 for the experiment (Figure 1). The planting pattern was summer peanut and winter wheat rotation. The soil type was Alfisol according to the Soil Taxonomy with clay texture and low pH value. The basic soil physiochemical properties are shown in Table 1.

Each field experiment was set up with two treatments, farmers' practice fertilization (FP) and optimized fertilization (OPT). All management of the FP treatment was determined by farmers based on their practices, and the rate of fertilizer applied was accurately recorded (Table 1). As a management strategy focused technology promotion, the determination of RMOR must be based on other approaches or data. Therefore, for the OPT treatment, the RMOR was determined based on the results of previous studies with similar environment, in which, the optimized amount of fertilizer was determined according to the yield–fertilizer rate response modeling [22]. In brief, a total of 46 field trials including four NPK fertilizer rates were collected. The average value of yield and fertilizer application under the same treatment was obtained. The yield–fertilizer rate response modeling was simulated by quadratic equation ($y = ax^2 + bx + c$, y: yield; x: N, P, or K fertilizer rates). Then the RMOR for NPK fertilizer was calculated at the maximum yield according to the modeling (-b/2a). The fertilizer recommendations were further adjusted by nutrient experts based on local conditions. The N, P, and K fertilizer rates for OPT in 2020 and 2021 were 181.5 kg/ha, 39.3 kg/ha, and 93.4 kg/ha, respectively (Figure S1). The other management measures were completely consistent with the FP treatment. Each treatment was repeated three times in plots of greater than 100 m^2.

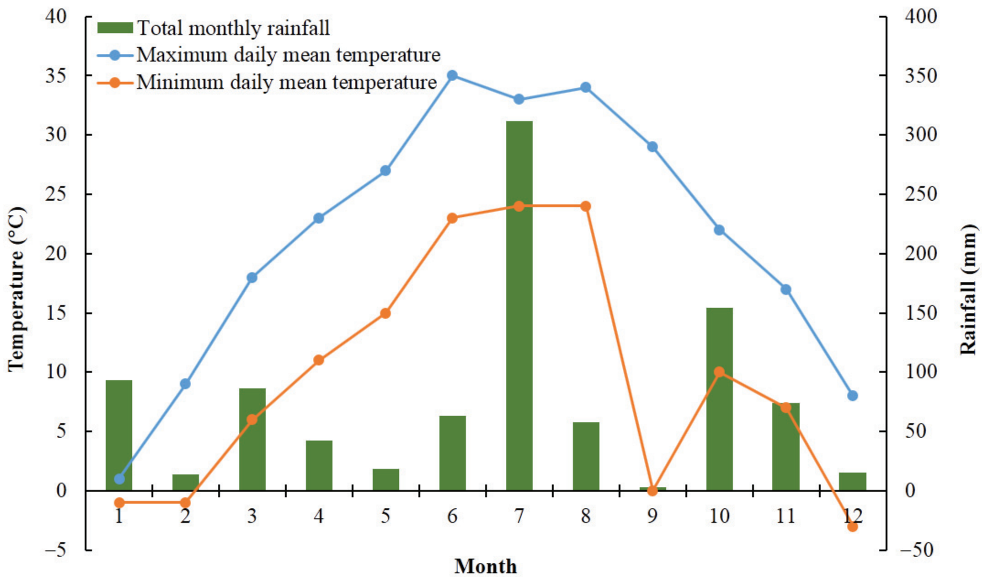

Figure 2. The monthly temperature and rainfall in Zhengyang County.

Table 1. Basic soil physiochemical properties and inputs of chemical fertilizers by the farmers' practice (FP) at different field sites in 2020 and 2021.

Year	Field Site	Soil Property at 0–20 cm Depth						Nutrient Inputs by Chemical Fertilizer		
		pH	SOC (%)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Olsen-P (mg/kg)	NH_4OAc-K (mg/kg)	N (kg/ha)	P (kg/ha)	K (kg/ha)
2020	A1	5.12	1.25	54.9	7.7	41.3	139.0	181.5	49.1	93.4
	A2	5.09	0.85	25.3	7.2	28.8	77.9	159.0	58.9	93.4
	A3	5.28	1.38	47.9	8.2	73.9	189.0	279.0	16.4	49.8
	A4	5.30	0.91	47.7	4.4	35.9	76.6	159.0	58.9	9.34
	A5	5.07	1.32	62.5	8.8	26.3	69.0	181.5	32.7	124.5
	A6	5.05	1.19	23.2	8.7	81.2	131.6	241.5	36.0	56.0
	A7	5.09	1.01	26.9	10.9	44.5	80.4	204.0	58.9	112.0
	A8	5.38	1.04	27.3	7.1	36.6	80.4	219.0	32.7	62.2
2021	B1	4.97	0.99	80.1	19.9	37.8	149.8	256.5	16.4	31.1
	B2	5.00	0.81	29.1	15.1	27.6	73.3	204.0	58.9	112.0
	B3	4.82	0.77	45.5	23.9	46.8	112.7	159.0	49.1	112.0
	B4	5.12	1.28	23.0	10.9	34.0	96.0	241.5	36.0	56.0
	B5	5.27	0.94	37.6	27.8	73.9	113.8	204.0	58.9	112.0
	Mean ± SD	5.12 ± 0.15	1.06 ± 0.21	40.8 ± 17.6	12.4 ± 7.0	45.3 ± 18.8	106.9 ± 36.7	206.9 ± 39.2	43.3 ± 16.0	85.2 ± 30.3

All peanuts were sown in early June and harvested at the end of September. The peanut varieties were decided by the farmers themselves, and they all used the widely grown varieties of momordica fruit type. Before peanut sowing, soil was plowed and then tilled. The peanuts were planted in ridges with a width of 80 cm at the bottom and 60 cm at the surface. Two rows of peanuts were planted on the ridge surface with a spacing of 20 cm. The overall sowing rate was 225 kg/ha. Ridging, sowing, and fertilizing were completed by mechanized methods performed once. Other management such as weed, pest, and disease control was consistent with FP treatment.

2.3. Sampling and Measurement

At peanut maturity, 2 m^2 peanut samples were taken for yield measurement in each plot of all experiment sites. The air-dried sample was weighed and its moisture content was determined, and further converted to the weight with a moisture content of 8%. Meanwhile, five representative plants were taken at random for dry matter (DM) and nutrient measurement. The haulm and pod were cleaned and sterilized at 105 °C for 30 min, then dried at 75 °C. After the plant and pod were dried to a constant weight, the DM weight (plant and pod) was recorded. The dried samples were crushed and passed through a 2 mm sieve for the determination of N, P, and K contents. A 0.2 g sample was digested with H_2SO_4-H_2O_2 to obtain a solution. The total N and P contents were determined using a flow injection analyzer (AA3, Seal, Germany), and the K content was determined by a flame photometer [23]. The fatty acid composition of peanut seeds was measured by the gas chromatography.

2.4. Data Processing and Analysis

Taking N as an example, parameters of NHI (N harvest index), RIE_N (N reciprocal internal efficiency), PFP_N (N fertilizer partial factor productivity), NUpE (N uptake efficiency), and NUtE (N utilization efficiency) were used to evaluate the characteristics of nutrient uptake and utilization [24,25]. The parameters were calculated as follows:

$$\text{NHI} = \text{N uptake in pod/total N uptake} \quad (1)$$

$$\text{RIE}_\text{N} = \text{total N uptake/pod yield} \times 1000 \quad (2)$$

$$\text{PFP}_\text{N} = \text{pod yield/N fertilizer rate} \quad (3)$$

$$\text{NUpE} = \text{total N uptake/N fertilizer rate} \quad (4)$$

$$\text{NUtE} = \text{pod yield/total N uptake} \quad (5)$$

The data were processed by Microsoft Excel 2016 software and graphed by Origin Lab Origin 2018 software. The statistical analysis of data were performed in SPSS 20.0 software. A one-way ANOVA was used for significant tests at $p < 0.05$.

3. Results

3.1. Dry Matter (DM), Pod Yield (PY), and Harvest Index (HI)

The PY ranged from 1958 kg/ha to 6915 kg/ha and averaged 4879 kg/ha for 13 field sites in 2 years. Compared with the FP treatment, OPT significantly increased PY in six field sites (Figure 3a). The yield increases by the OPT treatment ranged from -784 kg/ha to 1549 kg/ha and averaged 10.9%. The response of DM to different treatments was consistent with PY at 13 sites over 2 years (Figure 3b). Compared with FP, the DM increase in OPT ranged from −1179 kg/ha to 1544 kg/ha with an average rate of 6.6%. The variation range of peanut HI was 39.9%–64.0% with an average of 54.0% (Figure 3c). The peanut HI under OPT increased significantly in three field sites while decreasing significantly in other three field sites. There was no significant change in HI in total between FP and OPT treatments.

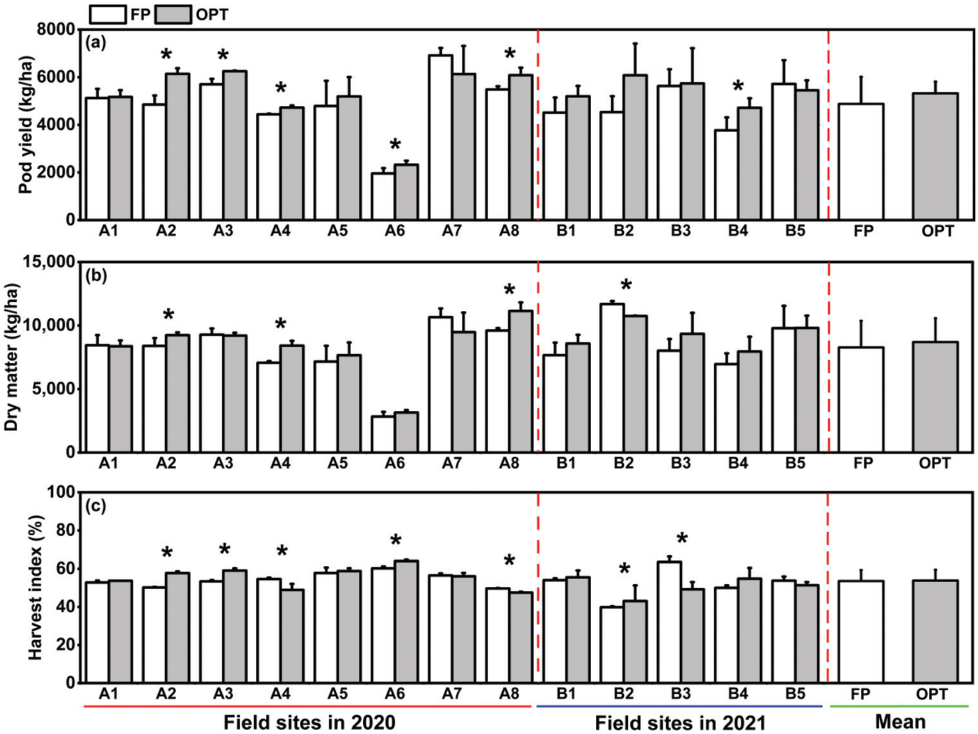

Figure 3. Variations in dry matter (DM, (**a**)), pod yield (PY, (**b**)), and harvest index (HI, (**c**)) of different fertilization treatment at different field sites in 2020 and 2021. FP: farmers' practice fertilization; OPT: optimized fertilization. A: experiment in 2021; B: experiment in 2022. Tentacle lines on the bars are standard deviation. Asterisks (*) on the bars indicate a significant difference at $p < 0.05$ between fertilization treatments for the same field site.

3.2. N, P, and K Uptake

There was significant variation in peanut nutrient uptake at different sites (Figure 4). For the two years, total N, P, and K uptake rates were 96.3–364.2, 6.2–42.6, and 23.0–110.3 kg/ha, and averaged 214.3, 23.3, and 78.4 kg/ha, respectively (Figure 4a,c,e). The total N, P, and K uptake rates under OPT were generally higher than in the FP treatment, with increasing rates of 19.3%, 7.3%, and 11.0%, respectively. In general, the N and P uptake rates by peanut straw were lower than for pods, while the K uptake was higher by straw than by pods, with a lower KHI (Figure 4f). The N, P, and K uptake rates were 164.3, 14.0, and 31.8 kg/ha in pods, and 50.0, 9.3, and 46.6 kg/ha in straw, respectively. For these 2 years, the NHI, PHI, and KHI were 61.7%-86.1%, 48.6%-74.7%, and 20.9%-60.7%, respectively, and averaged 76.0%, 59.8%, and 41.4%, respectively (Figure 4b,d,f). Although there were differences between FP and OPT in several field sites, in general, the OPT treatment did not significantly affect nutrient harvest indexes of peanuts in 13 field sites.

3.3. Reciprocal Internal Efficiency (RIE)

For 13 field sites in the 2 years, the RIE of N (RIE_N) averaged 42.0 kg with a range of 26.8–54.7 kg (Figure 5a). The averaged RIE_N was 6.9% higher under OPT than under FP. The RIE of P (RIE_P) in the 2 years varied from 2.7 kg to 7.5 kg with an average of 4.6 kg (Figure 5b). The RIE of K (RIE_K) varied from 11.1 kg to 19.9 kg with an average of 15.3 kg (Figure 5c). There was no significant difference in averaged RIE_K or RIE_P between FP and OPT treatments.

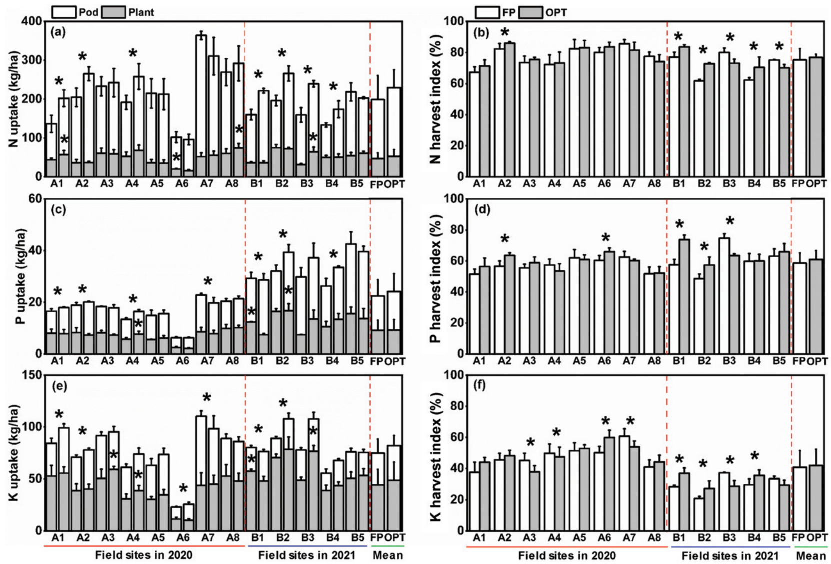

Figure 4. Uptake and nutrient harvest indexes of N (**a**,**b**), P (**c**,**d**), and K (**e**,**f**) in peanuts under different fertilization treatments. FP: farmers' practice fertilization; OPT: optimized fertilization. A: experiment in 2021; B: experiment in 2022. Tentacle lines on the bars are standard deviation. Asterisks (*) on the bars indicate a significant difference at $p < 0.05$ between fertilization treatments for the same field site.

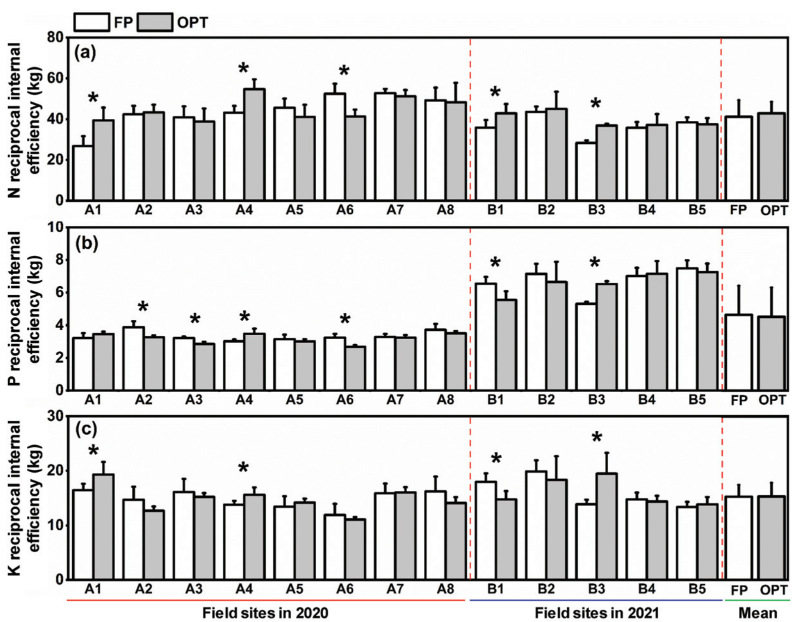

Figure 5. Requirements of N (**a**), P (**b**), and K (**c**) to produce 1000 kg pod yield of peanuts (reciprocal internal efficiency (RIE) under different treatments. FP: farmers' practice fertilization; OPT: optimized fertilization. A: experiment in 2021; B: experiment in 2022. Tentacle lines on the bars are standard deviation. Asterisks (*) on the bars indicate a significant difference at $p < 0.05$ between fertilization treatments for the same field site.

3.4. Partial Factor Productivity of NPK Fertilizer

Compared with the FP treatment, the average PFP_N of OPT increased by 15.1% in 2020 and by 29.8% in 2021 (Figure 6a). The average PFP_P of the OPT treatment decreased by 2.4% in 2020 and increased by 3.4% in 2021 compared with FP (Figure 6b). However, the average PFP_K of OPT decreased by 23.7% and 31.5% in the 2 years, respectively (Figure 6c).

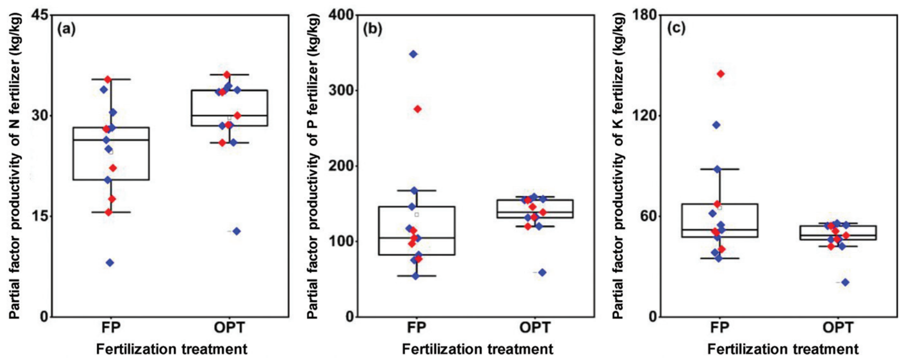

Figure 6. Partial factor productivity of N (**a**), P (**b**), and K fertilizer (**c**) under different fertilization treatments. FP: farmers' practice fertilization; OPT: optimized fertilization. For each boxplot, the top, middle, and bottom solid lines of the box represent the 75% quantile, 50% quantile, and 25% quantile, respectively; the top and bottom horizontal lines outside the box represent the 90% quantile, and 10% quantile, respectively. The square inside the box represents the mean. Blue or red diamonds: data in 2020 or 2021.

3.5. Nutrient Uptake Efficiency

There was a significant variation in NUpE among different field sites (Figure 7a). From 2020 to 2021, the average NUpE of the OPT treatment was increased by 19.3% and 45.2% compared with FP. The average PUpE of OPT decreased by 6.5% in 2020 and increased by 3.4% in 2021 compared with FP (Figure 7b). However, the average KUpE of OPT decreased by 25.5% and 32.2% compared with FP (Figure 7c).

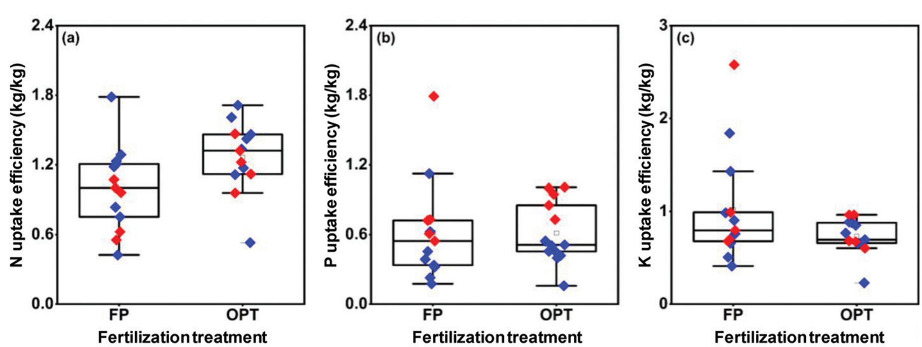

Figure 7. Uptake efficiency of N (**a**), P (**b**), and K (**c**) in peanuts under different fertilization treatments. FP: farmers' practice fertilization; OPT: optimized fertilization. For each box plot, the top, middle, and bottom solid lines of the box represent the 75% quantile, 50% quantile, and 25% quantile, respectively; the top and bottom horizontal lines outside the box represent the 90% quantile, and 10% quantile, respectively. The square inside the box represents the mean. Blue or red diamonds: data in 2020 or 2021.

3.6. Nutrient Utilization Efficiency

Compared with FP, the average NUtE for OPT was reduced by 3.8% in 2020 and by 11.7% in 2021 (Figure 8a). The average PUtE for OPT had a 4.8% increase in 2020 but an 11.7% decrease in 2021 compared with FP (Figure 8b). No clear difference in averaged KUtE between FP and OPT treatments was found in the 2 years (Figure 8c).

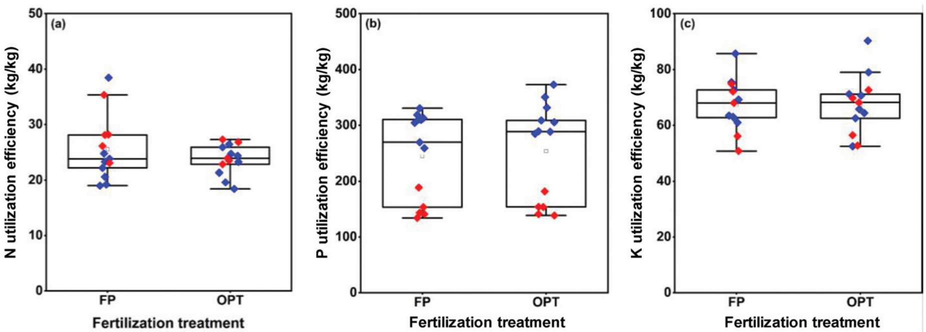

Figure 8. N (**a**), P (**b**), and K utilization efficiency (**c**) of peanuts under different fertilization treatments. FP: farmers' practice fertilization; OPT: optimized fertilization. For each box plot, the top, middle, and bottom solid lines of the box represent the 75% quantile, 50% quantile, and 25% quantile, respectively; the top and bottom horizontal lines outside the box represent the 90% quantile, and 10% quantile, respectively. The square inside the box represents the mean. Blue or red diamonds: data in 2020 or 2021.

3.7. Fatty Acid Composition of Peanut Kernels

In terms of the fatty acid composition of peanut kernels, oleic and linoleic acids accounted for 42.1% and 36.7%, respectively, followed by palmitic acid and stearic acid at 10.7% and 5.0%, respectively (Figure 9a). The ratio of oleic acid to linoleic acid (O/L ratio) was only 1.2 (Figure 9b). In general, the OPT treatment did not significantly affect the fatty acid composition or O/L of peanut kernels compared with FP.

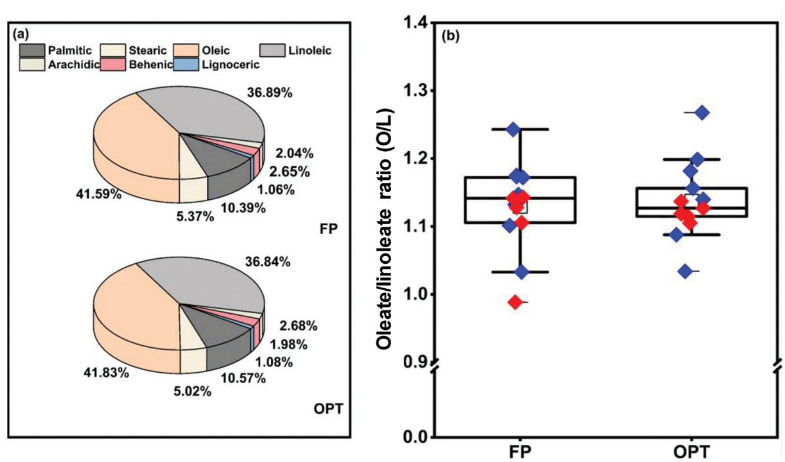

Figure 9. Fatty acid composition (**a**) and oleate/linoleate ratio (O/L, (**b**)) of peanut kernels under different fertilization treatments. FP: farmers' practice fertilization; OPT: optimized fertilization. For each box plot, the top, middle, and bottom solid lines of the box represent the 75% quantile, 50% quantile, and 25% quantile, respectively; the top and bottom horizontal lines outside the box represent the 90% quantile, and 10% quantile, respectively. The square inside the box represents the mean. Blue or red diamonds: data in 2020 or 2021.

4. Discussion

4.1. Feasibility of Fertilizer Recommendation Based on RMOR

There are some common concerns in developing countries such as small and scattered field sites, lack of test conditions, and lack of technical guidance for agrochemical services [15,26]. The present study demonstrated that the unified fertilization recommendations (i.e., RMOR) can achieve a reduction in chemical fertilizer and N use efficiency without yield loss in peanuts for smallholders in country scale. In these regions, this method has high practical value; the maximum benefit can be obtained, and the fertilizer recommendation is easy to promote. The theoretical basis of the RMOR method is to optimize the fertilizer application in the region scale, thus improving soil condition, the growth characteristics, and nutrient utilization of peanuts [22,27]. In this study, the N and P fertilizer rates according to RMOR were lower than the application rate in peanuts by the farmer practice in this region. Therefore, the simplified method can also save on N and P fertilizer. This is of great practical significance for decreasing the chemical fertilization rate while improving FUE, and thus decreasing the environmental risk, especially in areas with excessive chemical fertilizer consumption [28]. Once the RMOR for a specific crop in a certain region is determined, it can be applied for several years as long as there are no significant changes in climate or cultivation conditions. Therefore, the RMOR can be used as a basis for fertilizer development planning in the region, and as a basis for recommended fertilizer application rates for the crop in different field sites.

4.2. Limitation and Uncertainty

The RMOR is a management strategy for the optimization of regional fertilization, rather than a calculations method. Therefore, the determination of RMOR for a specific region needs to be based on other methods [19]. Meanwhile, applying the RMOR in the entire region, the benefits for individual farmers vary in this study; for some field sites, yields and nutrient efficiency even decrease, though it would not result in a significant yield loss for the region as a whole. The appropriate fertilizer rate for a given crop is affected by diverse factors such as management, soil, climate, and tillage [26,29]. This study showed that even within the same county there was significant variation in peanut yield and nutrient requirement. Therefore, the RMOR and its control range when applied should be delimited according to different conditions in specific regions and field sites and should be determined again with the changes in production environments. Furthermore, the adverse effects of the simplified practice can be decreased when combined with the specific conditions of each field, and then one can make appropriate adjustments of increase or decrease such as according to the soil fertility level, organic fertilizer application, and previous stubble [19,30]. Especially, for counties and regions with high requirements for precision fertilization, adjustment according to the specific situation of the local field and/or the necessary testing is needed to avoid the loss of yield and economic benefits from individual field sites.

4.3. Nutrient Requirements of Peanut

Compared with other studies with large sample sizes, the N requirement to produce 1000 pods in this study was similar to the result of 42.2 kg [25], but slightly lower than in other studies [31,32]; P and K demand was lower than what was estimated across China [31] and in the same region of central north China [32]. Estimation variation in nutrient requirement may be related to differences in planting regions, cultivars, soil conditions, yield level, and tillage [30,33]. Crucsiol et al. [3] demonstrated that N absorption for older cultivars remains high, while newer cultivars were less demanding in N. Xie et al. [25] found that the values of nutrient requirement simulated by modeling were lower than the average observed values, and they explained that the N, P, and K predicted by the model were the optimal nutrient requirements under the conditions of the balanced absorption of N, P, and K. However, high soil nutrient supply and excessive fertilization practices may have resulted in excess nutrient uptake [31]. A large number of studies have demonstrated that crop nutrient uptake positively correlates with yield level, but the yield increase decreases when the yield reaches a certain level [32]. As a result, nutrient requirements per unit yield tended to decrease as yields increased, especially when yields were above 70% of the potential yield [25]. The peanut yield has been strongly promoted in the past few decades in China due to cultivar renewal, adequate fertilization, soil improvement, and other management changes [32]. This means that the nutrient uptake requirements to produce unit pods may be decreasing.

5. Conclusions

The results of multi-site peanut experiments supported the hypothesis that compared with the FP treatment, the OPT treatment based on the RMOR method promoted N use efficiency (PFP_N and NU_PE), and decreased the nutrient inputs by chemical fertilizer, especially the N and P fertilizers, without the loss of peanut yield and NPK uptake. The NPK nutrient requirements of peanuts are quantified in this study, which is important for the regional recommendation of fertilization based on the RMOR. Therefore, the RMOR method is feasible for NPK fertilizer recommendations for peanut plantations, as it can simultaneously realize the optimization of agronomic, economic, and environmental benefits at a regional scale. The RMOR method can also be generally adopted in countries and regions with widespread smallholder farms. Furthermore, combined with precision fertilization technologies, the RMOR method is promising to realize agronomic and environmental optimization at the field scale.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants12061326/s1, Figure S1: the yield-fertilizer rate response modeling for the RMOR of NPK fertilizer.

Author Contributions: Conceptualization, Y.Z. and J.Y.; methodology, Y.Z., Y.L., and S.Z.; validation, J.H., Y.Y. (Yang Yang), H.Z., and Y.L.; formal analysis, J.Y. and Y.Y. (Youliang Ye); investigation, H.Z. and Y.H.; data curation, Y.Z. and J.Y.; writing—original draft preparation, J.H.; writing—review and editing, Y.Z., X.H., and J.Y.; visualization, J.H.; supervision, J.Y.; project administration, Y.Z. All authors have read and agreed to the published version of the manuscript.

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Article

Response of Normal and Low-Phytate Genotypes of Pea (*Pisum sativum* L.) on Phosphorus Foliar Fertilization

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Abstract: Phosphorus (P) is an important nutrient in plant nutrition. Its absorption by plants from the soil is influenced by many factors. Therefore, a foliar application of this nutrient could be utilized for the optimal nutrition state of plants. The premise of the study is that foliar application of phosphorus will increase the yield of normal-phytate (*npa*) cultivars (CDC Bronco a Cutlass) and low-phytate (*lpa*) lines (1-2347-144, 1-150-81) grown in soils with low phosphorus supply and affect seed quality depending on the ability of the pea to produce phytate. A graded application of phosphorus (H_3PO_4) in four doses: without P (P0), 27.3 mg P (P1), 54.5 mg P (P2), and 81.8 mg P/pot (P3) realized at the development stages of the 6th true leaf led to a significant increase of chlorophyll contents, and fluorescence parameters of chlorophyll expressing the CO_2 assimilation velocity. The P fertilization increased the yield of seeds significantly, except the highest dose of phosphorus (P3) at which the yield of the *npa* cultivars was reduced. The line 1-2347-144 was the most sensible to the P application when the dose P3 increased the seed production by 42.1%. Only the *lpa* line 1-150-81 showed a decreased tendency in the phytate content at the stepped application of the P nutrition. Foliar application of phosphorus significantly increased ash material in seed, but did not tend to affect the protein and mineral content of seeds. Only the zinc content in seeds was significantly reduced by foliar application of P in *npa* and *lpa* pea genotypes. It is concluded from the present study that foliar phosphorus application could be an effective way to enhance the pea growth in P-deficient condition with a direct effect on seed yield and quality.

Keywords: chlorophyll content; fluorescence parameters; seed yield; seed quality; seed nutrient content; pea; foliar application

1. Introduction

Pea (*Pisum sativum* L.) is a plentifully grown leguminous plant in many countries, and it can be utilized both in human nutrition as well as a part of the feed for farm animals. It is considered as one of the most important sources in human nutrition, because its pods contain a great content of proteins, carbohydrates, vitamins, and minerals. By its ability to hold nitrogen from the air and return it back to the soil, the pea contributes to sustainable agriculture [1]. Besides high demands for nitrogen, the pea belongs to those plants with relatively high demands for phosphorus (P), too.

Crop production on more than 30% of the world arable land is limited by the P availability [2]. P is an essential nutrient required by all plants to grow, photosynthesize, and form proteins. It is especially limiting in organic environments for legumes, which need more P than cereals to form root nodules for nitrogen fixation [3,4]. In particular, P is important for the growth of the field pea, and for protein synthesis [4]. Sufficient amounts of phosphorus taken by plants are also necessary for an optimal production yield. In the case of low P supplies in the soil or conditions decreasing its intake by plants, fertilization by phosphorus significant increases not only legume production, but also its quality [5–11]. One of the phosphorus forms naturally presented in plants is phytic acid. Phytic acid is the major storage form of phosphorus in seeds of legumes. In the case of the pea, its highest concentration is in the endosperm [12]. Since phytate can form complexes with proteins and minerals, reducing the digestive availability of phosphorus [13], it is usually regarded as an antinutrient, although recent works indicate that it has important beneficial roles as an antioxidant for plants [14]. Therefore, there is an interest in the assessment and manipulation of phytate contents in important food grains such as peas. Through plant breeding, it is possible to prevent the phosphorus transformation into phytic acid and, thus, to decrease the content of phytic acid by even up to 50–95% [15]. For example, Wilcox et al. [16] reported an 80% decrease in P-phytate content in the low-phytate soybean. Reduction of phytate phosphorus concentration in the low-phytate pea seed by about 60% accompanied by an increase in free inorganic phosphorus was presented by Warkentin [17]. The content of phytic acid in the pea is influenced not only by various properties, but also by the climate, irrigation, and soil conditions as well as by the fertilization. The decrease of phytate concentration caused by phosphorus fertilization was observed in the case of soya [18] oat [19] and corn [20].

One possible way to provide necessary nutrients to the plants during vegetative stages is a foliar application of fertilizers. The foliar fertilization directly applies the nutrient to the plant tissue, by-passing a potential fixation, and losses that may arise from the soil application. However, the efficacy of the foliar fertilizer is relatively uncertain [21]. An uptake potential of foliar P is generally considered to be low, but the increased efficiency of the P usage has been reported with foliar application [22]. The greatest benefits of the foliar P fertilization were observed under low moisture and highly P-deficient soil conditions. In these aforementioned conditions, the foliar P application has a potential to increase the yield and quality of seeds [23,24]. However, there have been relatively few reports on analyses of the productivity and seed quality of legumes [10] or normal [11] and low-phytate pea cultivars grown under varying foliar P fertilization levels.

This work contributes to the extension of knowledge on the foliar phosphorus application and its influence on selected growth parameters, production, and quality of normal-phytate cultivars, and low-phytate pea lines. It offers an alternative approach to optimize phosphorus nutrition of peas, i.e., the use of foliar fertilization with this nutrient, which is a suitable procedure especially in conditions where soil application seems ineffective (e.g., inappropriate soil pH, which immobilizes phosphorus in the soil). A positive effect of the foliar P application on pea seed production and its quality in plants grown under P deficient conditions, especially a reduction of the portion of P bounded to phytate, is expected. An increase of free P (inorganic) for nutritional purposes is expected. At the same time, it is determined whether the low-phytate lines will use the P supplied by fertilization directly for binding to phytate, i.e., will this increase the phytate phosphorus content or will it only increase the total P content of the seeds and the phytate level will remain unchanged?

2. Results and Discussion

2.1. The Effect of Foliar P Fertilization on Content of Chlorophyll and Chlorophyll Fluorescence Parameters

The foliar application of phosphorus had a significant effect ($p \leq 0.05$) on the content of chlorophyll in leaves of all tested pea genotypes. The increase of the N-tester values was significant ($p \leq 0.05, 0.01$) in both measurements, especially at variant P2 and P3. A significant effect of the foliar phosphorus application on the chlorophyll content of wheat was presented by Waraich et al. [25]. Besides wheat, the increased content of chlorophyll after the phosphorous fertilization was also achieved in the case of mung bean [26] aubergine [27] maize [28] and cluster bean [29]. The positive effect of the applied phosphorus can be explained by its direct involvement into the structure of cell membranes [30] a whole range of proteins, nucleic acids, and nucleotides [25] with direct effects on photosynthesis [31]. This fact explains the decrease of the chlorophyll content by the phosphorus deficiency in rice [32]. The mean N-tester values of all tested pea genotypes measured in the first term (T1) were enhanced by the stepped P application by 2.9% (P1), 4.2% (P2), and up to 4.9% (P3). The highest increase of the N-tester values was determined in plants of the line 1-150-81 at variant P3. The effect of the phosphorus fertilization lasted up to the second term of the measurement (T2), as presented in Table 1.

Table 1. The effect of the foliar phosphorus application on chlorophyll contents (N-tester value) and chlorophyll fluorescence parameters (Φ_{PSII} and R_{Fd}).

Genotype	Treatment	N-Tester Value		Φ_{PSII}		R_{Fd}	
		T1	T2	T1	T2	T1	T2
1-2347-144	P0	448 ± 6	378 ± 5	0.801 ± 0.011	0.809 ± 0.014	2.04 ± 0.03	1.94 ± 0.04
	P1	458 ± 5	390 ± 4 *	0.820 ± 0.008 *	0.825 ± 0.010	2.13 ± 0.02	2.06 ± 0.05 *
	P2	464 ± 7 **	401 ± 4 **	0.818 ± 0.007 *	0.831 ± 0.004	2.42 ± 0.20 **	2.07 ± 0.04 *
	P3	463 ± 6 *	402 ± 3 **	0.822 ± 0.002 *	0.833 ± 0.007 *	2.42 ± 0.20 **	2.13 ± 0.07 **
1-150-81	P0	443 ± 10	371 ± 3	0.792 ± 0.015	0.803 ± 0.004	1.99 ± 0.02	1.93 ± 0.07
	P1	456 ± 10 *	383 ± 7 *	0.816 ± 0.004 **	0.813 ± 0.005	2.08 ± 0.04	2.00 ± 0.07
	P2	464 ± 7 **	373 ± 9	0.816 ± 0.008 **	0.837 ± 0.005 **	2.11 ± 0.02	2.08 ± 0.06 *
	P3	475 ± 13 **	401 ± 7 **	0.829 ± 0.005 **	0.840 ± 0.007 **	2.16 ± 0.04 *	2.10 ± 0.04 **
Cutlass	P0	439 ± 6	368 ± 8	0.803 ± 0.011	0.792 ± 0.018	2.06 ± 0.04	1.77 ± 0.09
	P1	452 ± 5 *	374 ± 6	0.823 ± 0.008 *	0.792 ± 0.009	2.02 ± 0.03	1.82 ± 0.07
	P2	458 ± 5 **	402 ± 3 **	0.829 ± 0.005 **	0.815 ± 0.008	2.09 ± 0.06	1.97 ± 0.06 **
	P3	458 ± 6 **	381 ± 5 *	0.818 ± 0.019	0.789 ± 0.003	2.09 ± 0.02	1.76 ± 0.05
CDC Bronco	P0	447 ± 5	386 ± 8	0.804 ± 0.015	0.812 ± 0.043	2.00 ± 0.07	1.91 ± 0.04
	P1	463 ± 5 *	394 ± 12	0.828 ± 0.004 **	0.827 ± 0.006	2.05 ± 0.10	1.94 ± 0.07
	P2	465 ± 8 **	408 ± 4 **	0.825 ± 0.007 *	0.816 ± 0.004	2.22 ± 0.08 **	2.02 ± 0.08
	P3	468 ± 6 **	398 ± 6 *	0.820 ± 0.011 *	0.777 ± 0.008 **	2.07 ± 0.07	1.86 ± 0.13

The mean values marked with asterisk are significantly different (* $p \leq 0.05$; ** $p \leq 0.01$) from the variant without P fertilization (P0) by Fisher's LSD test (each of the genotypes was statistically evaluated separately). The values in the table represent the arithmetic mean (n = 60) ± SD (standard deviation). Determinations were carried out 14 (T1) and 28 (T2) days after the P-application.

The quantum yield of the electron transport of the photosystem II (Φ_{PSII}), which expresses the real capacity of the photosystem II (PSII) for photochemical reactions, and represents the availability of reaction centers of the PSII, was significantly ($p \leq 0.05$, 0.01) influenced by the phosphorus application. Its mean value measured at the variant without the P fertilization (P0) was 0.800. By the stepped P fertilization, this value was enhanced at all doses (P1−3) to 0.822 in the first term (T1). The results corresponded with conclusions in the study of Xu et al. [32], which found out that the phosphorus deficit had induced changes in efficiency of excitation energy absorption of reaction centers of the PSII in rice plants and had reduced the quantum yield Φ_{PSII}. For example, the decrease of the quantum yield (Φ_{PSII}) caused by the phosphorus deficit was observed in *Lonicera pampaninii* [33]. The photosynthetic rates in the *lpa* and *npa* genotypes of soybean were 1.3 and 1.5 times higher, respectively, in the treatment with the high P dose than with the low P dose [34]. In the first measurement term (T1), the Φ_{PSII} value was most significantly enhanced after the phosphorus application in the case of the line 1-150-81. According to Fryer et al. [35], a strong linear correlation between quantum yield of the electron transport of the PSII (Φ_{PSII}) and the carbon fixation efficiency was found. Plant species with phosphorus deficit like *Lotus japonicus* showed a decrease of the maximal rate of photosynthesis. In the case of the ratio of the dark respiration to the maximal photosynthesis, it declined significantly [36]. The significantly ($p \leq 0.05$; 0.01) increased Φ_{PSII} values of variants with the P fertilization on the *lpa* lines lasted until the later vegetation stages (T2). Contrary to this, the quantum yield values of the *npa* cultivars were reduced (Table 1). In the case of the cultivar CDC Bronco, the decrease was significant ($p \leq 0.01$). The increase of the Φ_{PSII} values at the *lpa* pea lines was followed by a significant enhancement of the fluorescence decrease ratio (R_{Fd}), which is measured at saturation irradiance, and which is directly proportional to the net CO_2 assimilation rate. In the second term T2, a significant correlation ($r = 0.635$; $p \leq 0.001$) between the Φ_{PSII} and R_{Fd} values was determined at these pea lines. Therefore, it can be stated that the foliar phosphorus application has a significant effect on the availability of reaction centers on the photosystem II at the *lpa* pea lines.

2.2. The Effect of Foliar P Fertilization, on Yield Parameters of Pea

The seed yield of normal phytate varieties was on average 27.3% higher than the seed yield of low-phytate lines. Research comparing yield levels of *lpa* and *npa* pea genotypes indicates that the low-phytate lines were similar in agronomic performance to normal phytate cultivars, except for somewhat slower time to flowering and maturity, slightly lower seed weight, and slightly lower grain yield [17,37]. However, the primary focus of our study was to evaluate the effect of phosphorus foliar fertilization on yield and seed quality of the tested pea genotypes.

The foliar application of phosphorus significantly influenced the yield of the pea seeds ($p \leq 0.05$; 0.01). Its enhancement was evident at all tested genotypes, as presented in Figure 1. A significantly increased grain yield was also achieved according to the literature dealing with the evaluation of the phosphorus application influence on legumes. The attention of contemporary research is mostly focused on the phosphorus uptake by the root system after the application of P-fertilizers in stepped doses to the soil. This application is used for the legumes growing both in a monoculture: pea [5], faba bean [6], chickpea [7], and mung bean [8], and in a mixed cultures [9]. Only a few literal sources evaluate the efficiency of the foliar P application on the seed production of legumes, and their quality [10,11]. Comparing the different techniques, the foliar P fertilization has a better potential to improve its nutritional deficiencies in plants caused by the low content of P in soil or limited availability of this nutrient by the root [23,38]. The reaction of the tested pea lines on the foliar P fertilization was different. In the case of the *npa* cultivars, the seed production was enhanced by the stepped doses of the P-fertilizer, except of the highest level (P3) which reduced the seed production. The yield was decreased by the mean value of 10% compared to the variant P2 at both tested cultivars. This fact corresponded to the parameters of photosynthesis determined during vegetation and indicated that the dose of 81.8 mg P (P3) is not beneficial to plants of the *npa* cultivars. Froese et al. [11] also observed a reduced yield caused by the highest dose of P (20 kg/ha P_2O_5) applied on leaves of pea. Contrary to the *npa* cultivars, the *lpa* line 1-2347-144 was positively affected by all fertilization variants, including P3, and provided significantly enhanced seed production compared to the variant P0. The stepped phosphorus application increased the production by 17.8% (P1), up to 22.2% (P3). The most significant effect of the foliar phosphorus application was achieved at the *lpa* line 1-150-81. In this case, the seed yield was also linearly increased by the applied phosphorus doses ($r = 0.849$; $p < 0.001$), and the highest level of the P fertilization (P3) induced the enhanced yield of 42.1%. The result corresponded to the response of the low-phytate lines of soyabean on the P fertilization, where the seed yield was significantly ($p \leq 0.05$) higher than at the normal-phytate genotypes [39]. However, these presented responses of pea on the foliar phosphorus fertilization were contradictory to the conclusions of Froese et al. [11]. In this study, the foliar P application was unable to substitute the seed-placed monoammonium phosphate and, overall, it had a marginal effect on the grain yield, P uptake as well as the seed nutritional value. According to the yield response on the phosphorus fertilization, the studied pea lines can be divided in two groups. While in the case of the *lpa* lines (1-2347-144; 1-150-81), the yield significantly correlated with the seed weight ($r = 0.597$, $p = 0.041$; $r = 0.752$, $p = 0.005$), in the case of the *npa* cultivars (CDC Bronco, and Cutlass), the pea production was significantly influenced by the number of seeds ($r = 0.828$, $p = 0.001$; $r = 0.600$, $p = 0.039$). According to the available literature, a positive influence of the foliar phosphorus application on the seed weight of common bean [39], chickpea [40], wheat, and maize [38] etc. was proved. Although the seed weight was relatively enhanced at all tested genotypes (Table 2), the influence of the phosphorus nutrition on the seed weight was significant ($p \leq 0.01$) only at the application of the highest P dose (P3) on the *lpa* line 1-150-81. The number of seeds produced by the pea plants was also influenced by the P nutrition. After the application of the P doses P2 and P3, the number of seeds was significantly increased ($p \leq 0.01$) at cultivars CDC Bronco and Cutlass by the mean 17.9% and 18.1%, respectively, compared to the non-fertilized variant (P0). In the case of the low-phytate lines, the number of seeds was also significantly ($p \leq 0.01$) increased by 8.0% and 13.0%, respectively, compared to P0 (Table 2).

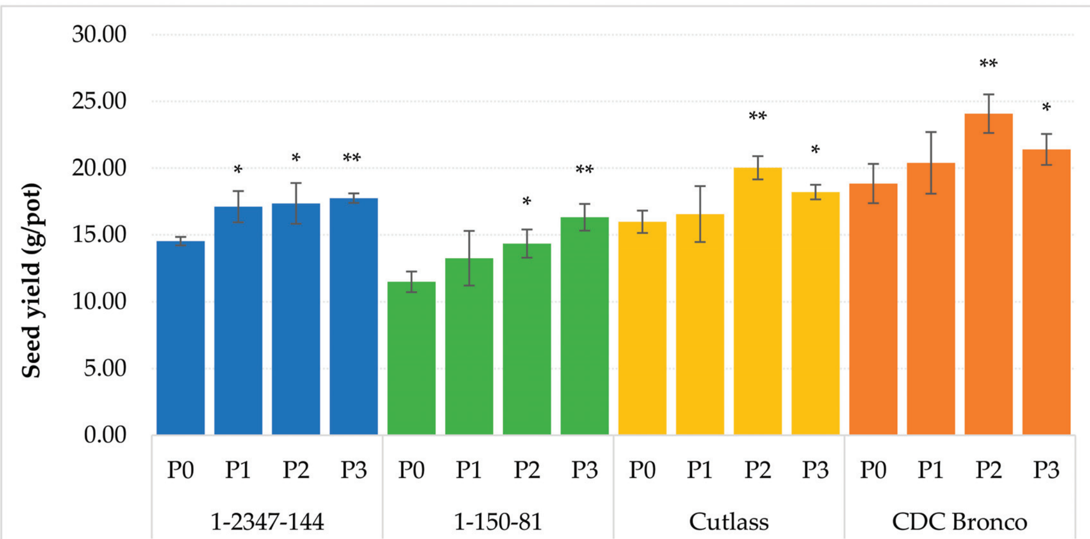

Figure 1. The effect of the foliar phosphorus application on the seed yield. The mean values (n = 8) marked with an asterisk are significantly different (* $p \leq 0.05$; ** $p \leq 0.01$) from the variant without the P fertilization (P0) by the Fisher's LSD test (each of the genotypes was statistically evaluated separately). Error bars represent the standard deviation of arithmetical mean (SD).

The foliar phosphorus application also significantly influence the height of plants, which correlated with the seed yield at the tested genotypes ($r = 0.786$, $p < 0.05$). While in the case of the *lpa* pea lines, the plant height was significantly ($p \leq 0.01$) increased even at the application of the highest phosphorus dose (P3), in the case of the cultivars CDC Bronco and Cutlass, the P3 dose did not influence the plant height. The cultivar Cutlass had no response on the P fertilization. By the mean values of the tested pea genotypes, the plant height was increased after the P fertilization by 3.4 (P1), 8.8 (P2), and 11.6 cm (P3) in the case of the low-phytate lines, and by 6.8 (P1), 7.0 (P2), and 1.4 cm (P3) in the case of the conventional cultivars (*npa*), compared to the control P0. According to the available literature, the phosphorus application to the soil had a significant influence on the height of cowpea [41]. Maize and wheat plant heights were also significantly increased by the foliar P application [38]. Interactions of the foliar P application with magnesium fertilization significantly increased the plant height of faba bean [10].

Table 2. The effect of the foliar phosphorus application on the plant height, seed weight and number of seeds.

Genotype	Treatment	Plant Height (cm)	Seed Weight (g/1000 Seeds)	Seed Number (Seed/Pot)
1-2347-144	P0	63 ± 5	193 ± 10	75.3 ± 3.8
	P1	66 ± 9	209 ± 20	81.7 ± 0.6 **
	P2	72 ± 5 *	220 ± 29	79.0 ± 2.0
	P3	75 ± 8 **	212 ± 16	84.0 ± 1.0 **
1-150-81	P0	64 ± 7	185 ± 10	62.3 ± 1.5
	P1	69 ± 7	197 ± 30	67.3 ± 2.5 *
	P2	73 ± 17 *	207 ± 26	69.3 ± 3.1 **
	P3	76 ± 9 **	229 ± 22 **	71.3 ± 1.5 **
Cutlass	P0	72 ± 2	188 ± 20	85.0 ± 4.4
	P1	75 ± 7	217 ± 7	76.3 ± 1.5 **
	P2	73 ± 4	212 ± 16	94.3 ± 4.5 **
	P3	71 ± 3	181 ± 16	100.7 ± 2.3 **
CDC Bronco	P0	98 ± 18	234 ± 16	80.3 ± 1.5
	P1	109 ± 11 **	238 ± 9	86.0 ± 3.6 *
	P2	111 ± 9 **	240 ± 10	100.3 ± 2.5 **
	P3	102 ± 9	226 ± 25	94.7 ± 1.5 **

The mean values marked with an asterisk are significantly different (* $p \leq 0.05$; ** $p \leq 0.01$) from the variant without P fertilization (P0) by the Fisher's LSD test (each of the genotypes was statistically evaluated separately). The values in the table represent the arithmetic mean (n = 8) ± SD (standard deviation).

2.3. The Effect of Foliar P Fertilization, on Pea Seed Quality

Within the seed, P is primarily stored as phytic acid and/or phytate that accumulate in protein vacuoles. Phytate comprises up to 80% of the total seed phosphorus, and can comprise as much as 1.5% of the seed dry weight [42]. Significantly, the lowest ($p \leq 0.05$) content of phytate in seeds was determined at the *lpa* line 1-150-81. By the P application, its content was reduced by 19.9% (P1), 24.9% (P2), and 9.1% (P3), respectively, but not significantly ($p \leq 0.01$). Nevertheless, the line 1-150-81 was the only one that tended to the decrease of phytate in seeds by the stepped phosphorus application. The foliar P application had a limited effect on phytate in seeds of canola, wheat, and pea [11]. In that experiment, phytate concentration in the pea seeds was decreased, comparing to the P non-fertilized variant, only by the application of the P-fertilizer (10 kg/ha P_2O_5) that was safely placed in the seed row with pea in combination with the foliar P application (10 kg/ha P_2O_5). The other tested genotypes, including the *lpa* line 1-2347-144, shown a relative increase of the phytate content by the stepped P application, significantly in the cultivar Cutlass, only (Table 3). It has been reported that the phytate concentration in seeds gradually increased and was positively correlated with the applied P levels in soybean [18], oat [19], and maize [20]. In context with the phosphorus content in seeds (Table 3), which was also significantly enhanced by fertilization only in the case of the cultivar Cutlass ($p \leq 0.01$), the portion of phytate-P amount to the total P content in seeds was evaluated for the studied genotypes (Figure 2). While in the case of the cultivar Cutlass, the fertilization by doses P2 and P3 increased the phytate-P portion from 65.6% (P0) to 73.4% and 73.7%, respectively, in the case of the line 1-150-81, a provable ($p \leq 0.05$) decrease of the phytate-P portion from 47.8% (P0) to 40.9% (P3) was induced by the foliar P nutrition.

Table 3. The effect of the foliar phosphorus application on the content of phosphorous, phytate, and crude protein in pea seeds.

Genotype	Treatment	P (% DM)	Phytate (g/100 g DM)	Crude Protein (% DM)
1-2347-144	P0	0.36 ± 0.02	0.82 ± 0.07	22.2 ± 0.4
	P1	0.35 ± 0.02	0.82 ± 0.04	23.4 ± 0.7
	P2	0.38 ± 0.06	0.90 ± 0.19	22.6 ± 0.4
	P3	0.41 ± 0.08	1.00 ± 0.27	23.3 ± 2.0
1-150-81	P0	0.42 ± 0.02	0.82 ± 0.22	23.7 ± 0.8
	P1	0.42 ± 0.04	0.66 ± 0.07	22.4 ± 2.0
	P2	0.39 ± 0.01	0.62 ± 0.04	20.9 ± 1.6 **
	P3	0.45 ± 0.02	0.75 ± 0.19	21.9 ± 0.4
Cutlass	P0	0.30 ± 0.01	0.69 ± 0.05	20.1 ± 0.5
	P1	0.30 ± 0.02	0.69 ± 0.06	21.1 ± 0.3
	P2	0.39 ± 0.03 **	1.01 ± 0.10 **	22.7 ± 1.1 **
	P3	0.43 ± 0.03 **	1.13 ± 0.08 **	24.1 ± 1.2 **
CDC Bronco	P0	0.41 ± 0.03	1.04 ± 0.10	20.0 ± 0.2
	P1	0.41 ± 0.03	0.98 ± 0.07	20.4 ± 1.1
	P2	0.41 ± 0.04	1.08 ± 0.07	20.7 ± 0.9
	P3	0.46 ± 0.03	1.14 ± 0.10	20.7 ± 0.8

The mean values marked with an asterisk are significantly different (** $p \leq 0.01$) from the variant without P fertilization (P0) by the Fisher's LSD test (each of the genotypes was statistically evaluated separately). The values in the table represent the arithmetic mean (n = 8) ± SD (standard deviation). (DM) dry matter.

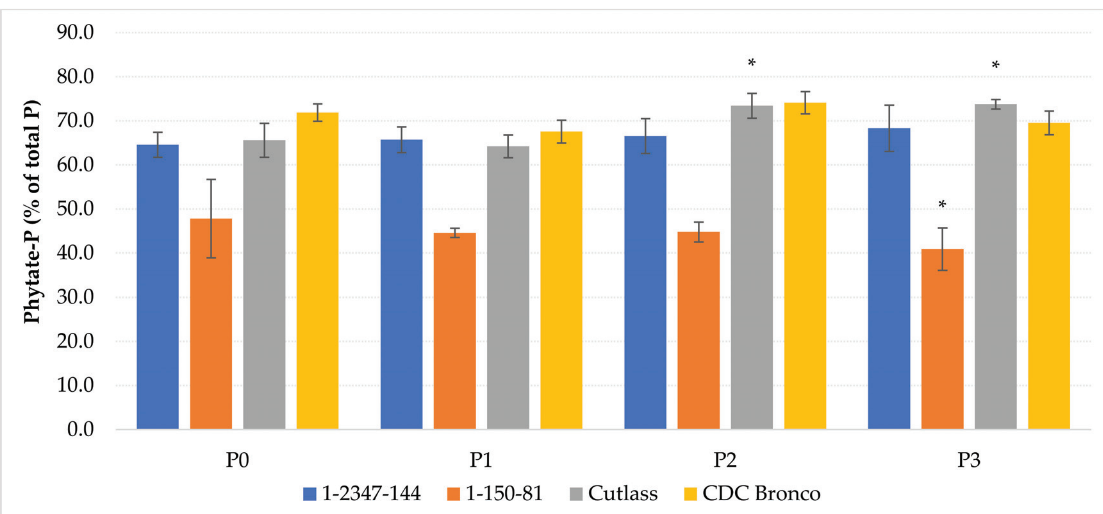

Figure 2. The effect of foliar phosphorus application on portion of phytate-P amount to the total P content in seeds. The mean values (n = 8) marked with an asterisk are significantly different (* $p \leq 0.05$) from the variant without P fertilization (P0) by the Fisher's LSD test (each of the genotypes was statistically evaluated separately). Error bars represent the standard deviation of arithmetical mean (SD).

The positive effect of the foliar phosphorus application on the crude protein content in the pea seeds was proved for the cultivar Cutlass, only (Table 3). In the case of the *lpa* line, content of the crude protein was not influenced by the P nutrition. The study by Klimek-Kopyra et al. [43] showed that phosphorus had a limited effect on this parameter. The phosphorus application in doses of 70 and 140 kg/ha P_2O_5, respectively, to the soil at 6 lines of pea did not influence content of the crude protein. Our study also does not confirm a consistent tendency towards the increase of the crude protein under higher phosphorus doses for cultivars of pea. Conversely, various *studies* [44,45] presented that the P fertilization increased the crude protein content of cowpea grain as well as low-phytate and normal-phytate cultivars of soybean [34].

The content of ash material and particular nutrients in the pea seeds of the tested genotypes is presented in Table 4. The relatively highest content of ash material was determined at the variant P3 for all tested genotypes. In this case, the mean increase of ash content was 0.13% compared to the variant P0. Besides the cultivar CDC Bronco, a significantly effect of the phosphorus application in doses P2 and/or P3 on the content of ash material was proved in tested genotypes. An important nutrient contained in the pea grain is potassium. Although its amount significantly correlated with the ash content ($r = 0.870$, $p < 0.05$), the foliar phosphorus application (P3) increased its content significantly only in the case of the line 1-2347-144 (Table 4). Potassium content was increased insignificantly by application of the highest dose of phosphorus (P3), from 0.92% to 0.98% DW on average across all tested pea genotypes. By contrast, the enhancement of potassium content in the tissue of the peanut plant observed after the phosphorus application was presented by Malakondaiach and Rajeswararao [46]. A significant increase in potassium uptake due to the increasing doses of phosphorus application was also found out in cowpea [47], mung bean [48], and urd bean [49]. The content of magnesium did not change by the P fertilization in seeds of the *lpa* lines, but it was increased in the *npa* cultivars (Table 4). A diverse response of the *npa* and *lpa* genotypes on the phosphorus fertilization was observed in the case of calcium utilization, too. While its content was not influence by the P fertilization of the cultivar CDC Bronco, it was significantly enhanced in the cultivar Cutlass. In the case of the low-phytate lines, Ca content was decreased, and in the case of the line 1-150-81 significantly. Since the effect of foliar P fertilization on seed mineral content in some genotypes is only statistically significant for some treatments, it is not possible to say that foliar P application will increase seed nutritive value.

Table 4. The effect of the foliar phosphorus application on ash content, and mineral content in pea seeds.

Genotype	Treatment	Ash (% DM)	K (% DM)	Mg (% DM)	Ca (% DM)	Zn (mg/kg DM)
1-2347-144	P0	2.89 ± 0.08	0.92 ± 0.02	0.125 ± 0.005	0.036 ± 0.005	34.0 ± 5.3
	P1	2.84 ± 0.06	0.85 ± 0.07	0.118 ± 0.011	0.041 ± 0.003	29.8 ± 3.2
	P2	2.96 ± 0.17	0.92 ± 0.05	0.121 ± 0.005	0.033 ± 0.003	25.8 ± 2.3
	P3	3.14 ± 0.22 *	1.01 ± 0.09 *	0.125 ± 0.009	0.030 ± 0.004	26.0 ± 2.3
1-150-81	P0	3.26 ± 0.13	1.02 ± 0.04	0.121 ± 0.008	0.040 ± 0.007	28.7 ± 0.6
	P1	3.13 ± 0.29	0.99 ± 0.09	0.114 ± 0.010	0.035 ± 0.003	28.5 ± 1.2
	P2	2.97 ± 0.06 *	0.95 ± 0.00	0.112 ± 0.006	0.032 ± 0.007 *	26.6 ± 5.8 *
	P3	3.09 ± 0.04	1.01 ± 0.00	0.121 ± 0.004	0.030 ± 0.004 **	25.6 ± 1.2 *
Cutlass	P0	2.68 ± 0.06	0.82 ± 0.06	0.109 ± 0.002	0.032 ± 0.002	24.7 ± 2.4
	P1	2.68 ± 0.07	0.83 ± 0.01	0.114 ± 0.009	0.033 ± 0.005	25.6 ± 4.0
	P2	2.96 ± 0.06 *	0.90 ± 0.03	0.116 ± 0.002	0.035 ± 0.005	25.6 ± 3.7
	P3	3.02 ± 0.18 **	0.87 ± 0.08	0.129 ± 0.007 **	0.045 ± 0.006 **	25.2 ± 1.6
CDC Bronco	P0	2.96 ± 0.14	0.93 ± 0.07	0.104 ± 0.004	0.030 ± 0.002	34.9 ± 3.7
	P1	2.96 ± 0.08	0.96 ± 0.03	0.111 ± 0.004	0.029 ± 0.002	34.4 ± 4.1
	P2	3.02 ± 0.11	0.96 ± 0.04	0.116 ± 0.007 *	0.029 ± 0.002	27.1 ± 2.4 *
	P3	3.08 ± 0.01	1.01 ± 0.01	0.108 ± 0.003	0.031 ± 0.005	29.1 ± 4.2

The mean values marked with an asterisk are significantly different (* $p \leq 0.05$; ** $p \leq 0.01$) from the variant without P fertilization (P0) by the Fisher's LSD test (each of the genotypes was statistically evaluated separately). The values in the table represent the arithmetic mean ($n = 8$) ± SD (standard deviation).

Zinc absorption capacity was reduced by the high phosphorus utilization, and zinc in the plant and soil was in a state of antagonism with phosphorus. According to the available studies, a deficiency of zinc in plants was caused by phosphorus fertilization to the soil [50,51]. The foliar phosphorus application significantly decreased the zinc content stored in seeds both in the *lpa* line (1-150-81) as well as in the *npa* cultivar (CDC Bronco).

One of possible explanations can be the fact that the increased P concentration in seeds due to the foliar phosphorous application can aggravate Zn deficiency. Another explanation is that zinc concentration in seeds of pea was decreased by the effect of the induced growth response on the P fertilization. In other words, zinc was diluted in the plant tissues. Thus, the foliar phosphorus application can decrease the nutrition value of the pea seeds.

The results of the pot experiment show the possibilities of using the foliar phosphorus fertilization in the growing of normal and low-phytate pea genotypes. It is shown that the foliar application increases not only the seed yield, but also their nutritional quality, usability in the food industry and human nutrition. It is clear that the phytate content of the seeds can be regulated in this way, which is variety dependent. A decrease in phosphorus binding to phytate was obtained in the low phytate line 1-150-81 and an increase in the Canadian variety Cutlass after foliar application of phosphorus. However, verification of the results obtained from the pot experiment in field trials will be necessary.

3. Materials and Methods

3.1. Plant Materials, Plant Cultivation and Conditions of Growth

The effect of the foliar phosphorus application on photosynthetic parameters, seed yields and quality of four pea genotypes (*Pisum Sativum* L.) was investigated in this study. The experiment was conducted in growth box (PlantMaster, CLF Plant Climatics GmbH, Wertingen, Germany) at the Mendel University in Brno located at 49°21′03′′ N and 16°61′38′′ E. The low-phytate pea lines (*lpa*) 1-150-81 and 1-2347-144 [17] were chosen for this study. In these lines, the content of phytate phosphorus is reduced by approximately 60% compared to the normal phytate genotype, with a compensating increase in inorganic phosphorus [17]. Both lines were derived from the cultivar CDC Bronco [52] through chemical mutagenesis [17]. Further, the normal-phytate cultivars (*npa*) CDC Bronco and Cutlass [53] were also used for this study. Pea plants were grown under the control condition: 12 h of light (light intensity 550 µmol m^{-2} s^{-1}) and 12 h of darkness; day temperature 22 °C and night temperature 15 °C; humidity 60% during the day and 90% at night. Mitscherlich pots with a volume of 6.2 L were used to grow the pea plants. Each container contained 6000 g of arable soil with a constant composition (Table 5).

Table 5. Chemical composition of soil used in this study.

Soil Parameter	Value
pH ($CaCl_2$)	6.09
Soil oxidizable carbon (Cox)	0.80%
Clay	20%
Silt	27%
Sand	53%
CEC (Cation Exchange Capacity)	164 mmol/kg
total N	0.19%
Ammonium N (NH_4^+)	1.48 mg/kg
Nitrate N (NO_3^-)	17.2 mg/kg
Available P (Mehlich III)	36.4 mg/kg *
Available K (Mehlich III)	400 mg/kg
Available Ca (Mehlich III)	2 720 mg/kg
Available Mg (Mehlich III)	214 mg/kg

* low available phosphorus content. Soil parameters were determined according to Zbíral [54].

Six pea seeds of each genotype per pot were sown on 27 April 2020. Four plants were grown in each pot for all pea genotypes. The foliar fertilization of phosphorus was carried out on the plant development stages of the 6th true leaf unfolded at the 6th node (3 June 2020). The following four treatments with three doses of the foliar phosphorous application were included in the experiment: P0—without P (0 mg P/pot), P1—62.5 mg P_2O_5 (27.3 mg P) per pot; P2—125 mg P_2O_5 (54.5 mg P) per pot and P3—187.5 mg P_2O_5 (81.8 mg P) per pot. These treatments of the foliar phosphorus application were carried out for all genotypes (for each genotype separately). The phosphorous foliar application (phosphoric acid, H_3PO_4) in 5 mL of water solution per pot at each treatment (P1−P3) was used. The control variant (P0) was treated with water. Water and phosphorus solutions were regularly applied using a pressurized hand pump sprayer (DPZ 1500, ProGlass, Weilheim an der Teck, Germany). The vegetation pots were arranged randomly in the growth box. A total of 32 pots were established for each genotype: four treatments with the graded dose of P (P0–P3); each treatment was established in eight replications (pots).

3.2. Measurement of Photosynthetic Parameters of Pea Plants

Selected photosynthetic parameters of pea plant The content of chlorophyll (like N-tester value), and selected parameters of fluorescence (quantum yield of photosystem II, and chlorophyll fluorescence decrease ratio) were evaluated. The measurements were performed 14 (T1) and 28 days (T2) after the foliar application.

3.2.1. Content of Chlorophyll (N-Tester Value)

The content of chlorophyll in pea leaves, expressed as N-tester values, was determined using an N-Tester instrument (Yara International ASA, Oslo, Norway) in the wavelength range 650–940 nm [55]. Chlorophyll content was determined from leaves located in the middle part of the plants.

3.2.2. Chlorophyll Fluorescence Parameters

Photochemical *efficiency* of *photosystem II* (PSII) in pea plants was determined. Chlorophyll fluorescence determination was performed with a PAR-FluorPen FP 110-LM/S (Photon Systems Instruments, Drásov, Czech Republic). The measured data were subsequently evaluated using FluorPen 1.1 software [56]. The leaves of the pea plant were dark adapted for 30 min prior to the measurement. Protocol for the measurement of chlorophyll fluorescence parameters is presented in Table 6.

Table 6. Measurement protocol of the chlorophyll fluorescence.

Chlorophyll Fluorescence Parameters	Pulse Type	Light Intensity (µmol/m^2/s)	Phase	Duration (s)	1st Pulse (s)	Pulse Interval (s)
Φ_{PSII}	Saturation	2400	-	1 pulse		
R_{Fd}	Flash	900	L	60	0.2	1
			DR	88	1	1
	Saturation	2400	L	60	7	12
			DR	88	11	26
	Actinic	300	L	60	-	-

λ = 454 nm, L—light, DR—Dark recovery.

The Quantum yield of the PSII (Φ_{PSII}) and the Chlorophyll fluorescence decrease ratio (R_{Fd}) were measured as a photochemical quenching parameter (Table 7).

Table 7. The photochemical quenching parameter.

Chlorophyll Fluorescence Parameters		Ref.
Φ_{PSII}	$F_m - F_0/F_m$	[57]
R_{Fd}	F_d/F_s	[58]

(F_0) minimal fluorescence from the dark-adapted leaves, F_m means maximal fluorescence from the dark-adapted leaves; (F_d) fluorescence decrease from (F_m to F_s; F_s) steady state chlorophyll fluorescence.

3.3. Yield Parameters and Seed Quality

The pea plants were harvested at the full ripeness on 29 July 2020. The height of plants, the yield of seeds per pot, the weight of one thousand seeds, and the number of seeds were determined. Plant height was measured before harvesting. After cutting the plants, the pea seeds were harvested by hand. The pea seeds were then weighed (laboratory scale PCB Kern, KERN and Sohn GmbH, Balingen, Germany), and counted (seed counter Contador, Pfeuffer GmbH, Kitzingen, Germany). The weight of 1000-seeds was subsequently determined.

Subsequently, the pea seeds were analyzed in order to evaluate selected qualitative parameters, namely: the content of phytic acid (phytate), content of phosphorus in the phytate, content of crude protein, ash material, and content of nutrients in seeds (P, K, Mg, Ca, and Zn).

3.3.1. Determination of Phytic Acid (Phytate)

Content of phytic acid was determined using a commercial kit „Phytic acid (phytate)/Total phosphorus" (Megazyme, Bray, Ireland) [59]. Pea seeds were finely ground using the Foss Tecator Cyclotec 1093 (Foss Analytical, Hillerød, Denmark). For analysis, 1 g of flour was weighted. Phytic acid from the sample was extracted with 0.66 M HCl. The neutralized aliquot of the sample was treated with phytase that was specific for phytic acid, and the lower myo-inositol phosphate forms. Then, the sample was treated with alkaline phosphatase that hydrolyzed myo-inositol phosphates and released free phosphate. The total phosphate was measured using a colorimetric method with molybdenum blue (Spekol 1300, Analytik Jena AG, Jena, Germany). The amount of molybdenum blue was proportional to the amount of phosphate in the sample.

3.3.2. Analysis of Crude Protein and Ash

Ash material was determined by weighing the material remaining after the burning of a fixed-weight sample at 550 °C and specified conditions. Nitrogenous substances were measured by the Kjeldal method (N $\times$ 6.25 coefficient) using the Kjeltec 2300 device (Foss Analytical, Hillerød, Denmark) [60].

3.3.3. Determination of Nutrient Contents

The samples of pea seeds were dried at temperature of 50 °C, then crushed in the grinder (Foss Tecator Cyclotec 1093, Foss Analytical, Hillerød, Denmark), and homogenized. After the microwave closed vessel acid digestion (HNO_3/H_2O_2) in ETHOS One (Milestone Srl, Sorisole, Italy), the contents of nutrients were determined (Table 8).

Table 8. The methods for the determination of nutrients in pea seed.

Nutrient	Method Used	Device Used	Ref.
P	Spectrophotometry	Unicam 8625 UV/VIS (Pye Unicam Ltd., Cambridge, UK)	[61]
K, Mg, Ca, Zn	Atomic absorption spectrometry	ContrAA 700 (Analytik Jena AG, Jena, Germany)	[61]

(UV/VIS) ultraviolet–visible.

3.4. Statistical Data Analysis

Measured data were statistically evaluated using STATISTICA 12 program (TIBCO Software Inc., Palo Alto, CA, USA) [62]. The normality and homogeneity of variances were verified, respectively, by Shapiro-Wilk's and Levene's test at $p \leq 0.05$. The influence of the monitored factors was analyzed via two-way ANOVA (level of significance $p \leq 0.05$). All evaluated parameters are expressed in tables and graphs as the arithmetic mean $\pm$ standard deviation (SD). The differences between the arithmetical means were evaluated by the Fisher's (*LSD*) test at the 95% ($p < 0.05$), and 99% ($p < 0.01$) level of significance.

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Using Waste Sulfur from Biogas Production in Combination with Nitrogen Fertilization of Maize (*Zea mays* L.) by Foliar Application

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Abstract: In Europe, mainly due to industrial desulfurization, the supply of soil sulfur (S), an essential nutrient for crops, has been declining. One of the currently promoted sources of renewable energy is biogas production, which produces S as a waste product. In order to confirm the effect of the foliar application of waste elemental S in combination with liquid urea ammonium nitrate (UAN) fertilizer, a vegetation experiment was conducted with maize as the main crop grown for biogas production. The following treatments were included in the experiment: 1. Control (no fertilization), 2. UAN, 3. UANS1 (N:S ratio, 2:1), 4. UANS2 (1:1), 5. UANS3 (1:2). The application of UAN increased the N content in the plant and significantly affected the chlorophyll content (N-tester value). Despite the lower increase in nitrogen (N) content and uptake by the plant due to the application of UANS, these combinations had a significant effect on the quantum yield of PSII. The application of UANS significantly increased the S content of the plant. The increase in the weight of plants found on the treatment fertilized with UANS can be explained by the synergistic relationship between N and S, which contributed to the increase in crop nitrogen use efficiency. This study suggests that the foliar application of waste elemental S in combination with UAN at a 1:1 ratio could be an effective way to optimize the nutritional status of maize while reducing mineral fertilizer consumption.

Keywords: chlorophyll content; fluorescence parameters; plant weight; plant nutrient content; nitrogen use efficiency

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1. Introduction

One of the principles of the European Green Deal is the proposal of greenhouse gas emissions cut by at least 55% by the year 2030, which should set Europe to a path to becoming climate-neutral by the year 2050 [1]. According to the European Biogas Association (EBA), biogas, biomethane, and other renewable gases will play a key role in helping Europe's transition to a clean energy system [2], and the European Commission's strategies promise targeted support for biogas in the revised Renewable Energy Directive and gas legislation. EBA, Eurogas, and the Gas for Climate consortium are calling for an EU-wide renewable target of at least 11%. The annual production of biogas in Europe reaches 15.8 bcm and is relatively stable with a total of 18,943 biogas plants according to the EBA [3].

A biogas plant produces biogas, which can then be used for the cogeneration of electricity and heat. Biogas is a mixture of methane, carbon dioxide, and other components such as hydrogen sulfide (H_2S) [4]. The biogas must be pretreated before use. The first step of the purification process is the removal of H_2S, which is corrosive and harmful to health [5,6]. Biogas production is thus associated with the production of waste products.

The utilization of waste sulfur obtained from the purification process seems to be promising from the point of view of plant nutrition and especially from the economic aspect of biogas production [7,8] and sulfur deficiency in the environment.

European SO_2 emissions have been reduced by 70–80% since 1990 [9,10]. According to results of Engardt et al. [11], sulfur deposition in Europe will decrease until, at least, 2050. For example, in the Czech Republic, atmospheric sulfur deposition is about 5 kg/ha per year [12], so there is a shortage of sulfur in the soil, as it has been presented by many authors [13–18]. According to Zbíral et al. [19], a statistically highly significant decrease in the soil S content caused by reduction of SO_2 emissions in the long-term field experiments in Czech Republic from 33 mg/kg in 1981 to 8 mg/kg in 2017. Therefore, it is necessary to pay special attention to fertilization by sulfur in addition to the other essential nutrients, especially because of the increased cultivation of crops with high sulfur requirements [19,20]. Sulfur in plants is essential for the synthesis of cysteine, methionine, and some vitamins [21]. The deficiency of sulfur in maize as the main crop for biogas production not only reduces yield but also quality parameters such as the content of starch, carbohydrates, and proteins [22]. Sulfur is usually applied in the form of mineral fertilizers, and co-application with nitrogen is recommended by many authors as these nutrients have been proven to have good synergy [23,24]. Salvagiotti and Miralles [25] showed that S addition increased the biomass and grain yield of cereal and the positive interaction of N and S, which resulted in a greater nitrogen use efficiency. A shortage of S supply also lowers the utilization of nitrogen and results in a deterioration in crop quality [26]. As sulfur is an essential constituent of enzymes involved in nitrogen metabolism, its deficiency could lead to a decrease in N assimilation [27,28]. Some reports have shown the accumulation of nitrates in S-deficient plants [29]. In addition, Haneklaus et al. [30] reported that each kg of S deficit causes 15 kg of nitrogen to be lost in the environment. Maize is an important crop that, despite its relatively low sulfur requirements, is severely affected by its deficiency [31,32].

Nitrogen is essential for plants in terms of biomass and yield production [33]. In addition to the conventional nitrogen fertilization of the soil, the nutritional status of the plants can be optimized by foliar fertilization during the plant growth [34]. Foliar fertilization could be used under farming conditions as a quick correction for unexpected nutrient deficiencies, for the late supply of N (and another nutrients) during advanced growth stages, and as a preventive measure against unsuspected (or hidden) deficiencies [35–38]. The foliar application of nutrients is also recommended when the soil or the plant conditions limit the availability of some nutrients [39] and is appropriate under conditions when high loss rates of soil-applied nutrients may occur [40]. For example, the foliar application of nitrogen has significantly improved the grain yield of maize [41] and other cereal crops [42].

The aim of this study was to verify the effect of the foliar application of waste elemental sulfur from biogas production in combination with conventional liquid fertilizers UAN applied in different ratios. Such a reutilization of waste sulfur from biogas plants back in agriculture is suitable from the economic aspect of biogas purification and waste management. The application of this sulfur could help to reduce the consumption of mineral fertilizers and, at the same time, address the deficient sulfur content in the soil and plants.

2. Results and Discussion

The application of UAN fertilizer alone and in combination with sulfur increased the chlorophyll content (N-tester value) in maize leaves compared to the unfertilized control. The increase in chlorophyll was evident at both monitoring terms (t1 and t2), while the differences between the control (N-unfertilized treatment) and the N (UAN) and NS (UANS1-3)-fertilized treatments increased over time (Table 1).

Table 1. The effect of the foliar fertilizer application on chlorophyll contents (N-tester value).

Treatment	t1		t2	
	N-Tester Value	Rel. %	N-Tester Value	Rel. %
Control	271 ± 5^{f}	100.0	196 ± 12^{g}	100.0
UAN	465 ± 11^{a}	171.6	379 ± 13^{cd}	193.4
UANS1	397 ± 14^{bc}	146.5	349 ± 28^{de}	178.0
UANS2	414 ± 11^{b}	152.8	325 ± 12^{e}	165.8
UANS3	393 ± 12^{bc}	145.0	319 ± 13^{e}	162.8

The values in the table represent the arithmetic mean (n = 8) ± SD (standard deviation). The same letters next to the numbers describe no statistically significant differences between the treatments (Fisher's LSD test, $p < 0.05$). The relative expression of the values is shown in the column marked Rel. % (Control = 100%). The measurements were performed at two growth stages, t1 (5th true leaf) and t2 (6th true leaf).

The N-tester values were significantly correlated with the rate of nitrogen applied in fertilizers at both terms, as presented in Figure 1. The results agree with several studies that have reported a strong correlation between chlorophyll content and the amount of nitrogen in leaves [43–47].

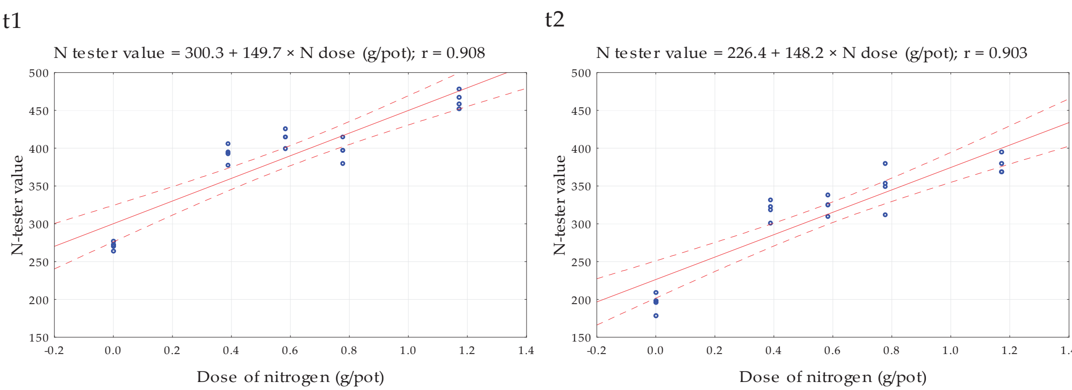

Figure 1. Dependence of N-tester value on nitrogen dose. The measurements were carried out on the 1st (t1) and 2nd (t2) growth stages of maize.

Nitrogen is part of the enzymes associated with chlorophyll synthesis [48] and the chlorophyll concentration reflects relative crop N status. Statistically significant highest N-tester values were found for the treatment fertilized with UAN applied without sulfur (UAN) on both measurement terms (t1; t2). The highest nitrogen dose was applied on this treatment. The application of UAN in combination with elemental sulfur (UANS1–3) significantly increased the N-tester value compared to the unfertilized (control) treatment, but the level of chlorophyll content did not reach the values found in plants fertilized with UAN alone. While, in the first measurement term (t1), the highest N-tester value was found for the UANS2 treatment (Table 1), in term t2, the N-tester values were in direct dependence on the nitrogen doses contained in the UAN–sulfur mixture. The N-tester values found at both terms (t1; t2) were significantly correlated with the plant nitrogen content detected at term t3 (r = 0.711, $p < 0.001$; r = 0.707, $p < 0.001$, respectively). Evaluation of the nutritional status after the joint application of nitrogen and sulfur using the N-tester was also performed on several dates by Lacroux et al. [49], and their results showed a significant increase in measured values compared to the control, with the highest values achieved by the joint foliar application of N and S.

The ability of the photosystem II to absorb radiation is expressed by the variable chlorophyll fluorescence for dark-adapted leaves (F_v). The more radiation a plant can absorb, the more radiation the plant can use for photosynthesis. Although the ability of the plant tissue to absorb radiation decreased over time (comparison of F_v levels between t1 and t2), this decrease was not significant for the UAN and UAN combination with sulfur. A significant reduction in F_v values was only observed in the unfertilized treatment (Figure 2). Even though the treatment with the highest sulfur dose (UANS3) showed the lowest F_v values, the results showed that the decrease in F_v between terms t1 and t2 was smallest on this treatment. Nitrogen deficiency decreases the photosynthetic assimilation capacity of CO_2 of plant leaves, leading to decreases in light-saturated photosynthetic rates [50]. In addition, Ciompi et al. [51] and Jin et al. [52] reported a positive correlation between the nitrogen content in the plant tissue of leaves and photosynthetic capacity.

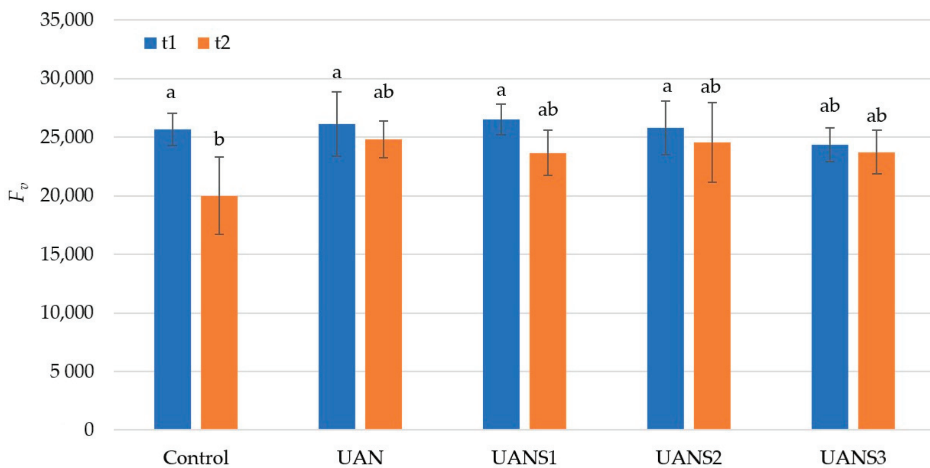

Figure 2. Variable fluorescence (F_v) value after the foliar application of fertilizers. The measurements were carried out on two growth stages of maize (t1 and t2). The values represent the arithmetic mean (n = 8); the bars represent the standard deviation of the mean. There are no statistical differences between columns with the same letters (Fisher's LSD test, $p < 0.05$).

After dark adaptation of the maize leaves, the maximum photosynthetic capacity (Φ_{PSII}) was estimated as the quotient between variable and maximum fluorescence (F_v/F_m). The quantum yield, which indicates the actual capacity for photochemical processes by the availability of reaction centers of the photosystem II (PSII), was significantly ($p \leq 0.05$) influenced by the fertilizer application (Figure 3). It is clear that nitrogen significantly affects photosynthesis and chlorophyll fluorescence of the plant. This was demonstrated by the response of maize to nitrogen fertilization in a study by Ahmad et al. [53], in which the effect of nitrogen application increased the electron transport rate, photochemical quenching coefficient, variable fluorescence, maximal quantum yield, and effective quantum yield of PSII photochemistry. A significant increase in Φ_{PSII} values in three maize varieties due to a high nitrogen dose was demonstrated by Jin et al. [52]. Reductions in the quantum yield of PSII electron transfer due to nitrogen deficiency were also described by Nunes et al. [54] and Verhoeven et al. [55]. In our study, the values of Φ_{PSII} were decreased over time regardless of fertilization treatment. The highest value of Φ_{PSII} was determined after the application of UAN with the highest elemental sulfur content (UANS3). These results contradict the above studies, but, on the other hand, they show a positive effect of applied sulfur on nitrogen utilization and its use by the plant. A high linear dependence between the efficiency of carbon fixation and quantum yield value was presented by Fryer et al. [56].

The rate of fluorescence decline (R_{Fd}), an empirical parameter for the quantification of plant vitality under tested conditions, was measured. In contrast to the values of the variable chlorophyll fluorescence (F_v) and quantum yield of PSII (Φ_{PSII}), the rate of fluorescence decline was not statistically significantly affected by foliar fertilization. Only at term t2 did the R_{Fd} value of plants grown on the UANS2 treatment decrease significantly below the control level, but no trend in the decrease in R_{Fd} due to UAN fertilization in combination with elemental sulfur was observed (Figure 4).

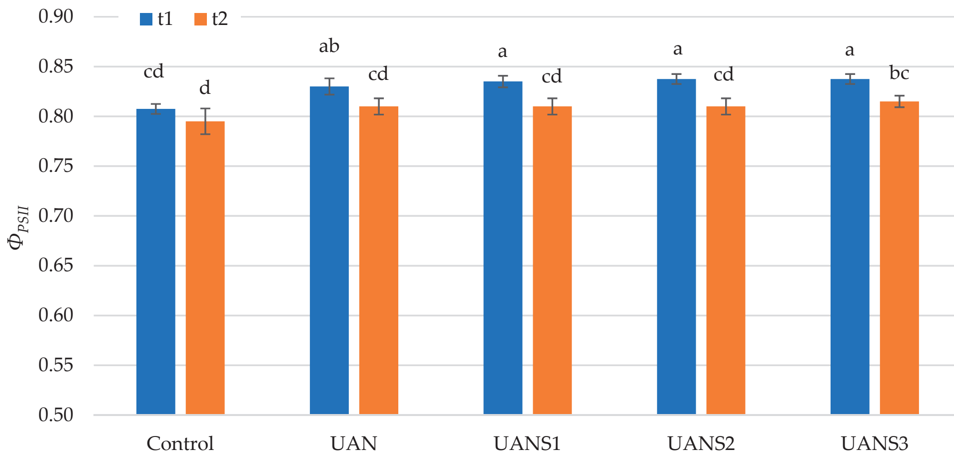

Figure 3. The effect of the foliar application of fertilizers on the quantum yield of PSII photochemistry (Φ_{PSII}). The measurements were carried out on two growth stages of maize (t1 and t2). The values represent the arithmetic mean ($n = 8$); the bars represent the standard deviation of the mean. There are no statistical differences between columns with the same letters (Fisher's LSD test, $p < 0.05$).

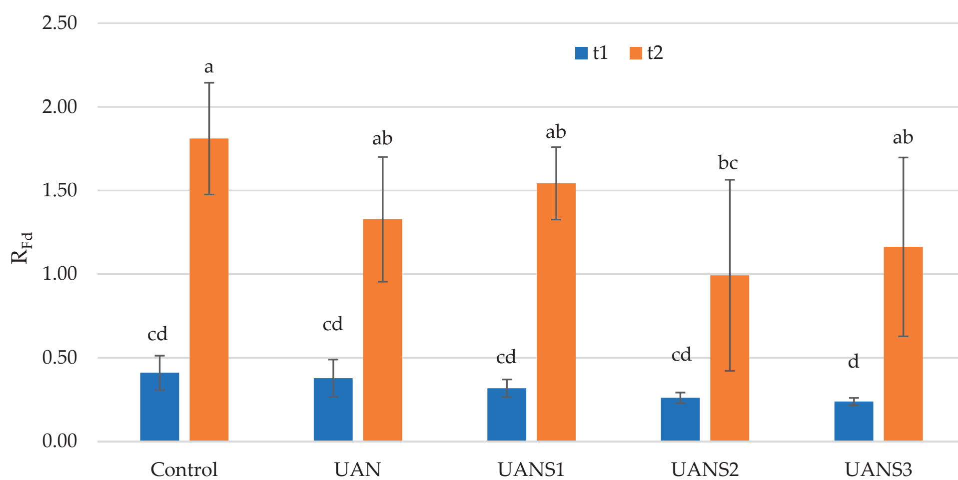

Figure 4. Fluorescence decrease ratio (R_{Fd}) in maize leaves after the foliar application of fertilizer. The measurements were carried out on two growth stages of maize (t1 and t2). The values represent the arithmetic mean ($n = 8$); the bars represent the standard deviation of the mean. There are no statistical differences between columns with the same letters (Fisher's LSD test, $p < 0.05$).

The average dry weight of the above-ground biomass (AGB) of plants determined on the 35th day after the foliar application of fertilizer (t3) is shown in Figure 5. The highest plant dry weight was found for the treatment fertilized with UAN, which provided the most nitrogen to the plants. The dry weight of plants produced on this treatment was 2.4 times higher compared to the unfertilized Control. The dry weight of plants fertilized with the UANS fertilizer combination ranged from 17.44 to 17.84 g/plant and was not statistically different from the UAN treatment (Figure 5). A significant effect of foliar nitrogen application on plant dry matter yield has been demonstrated in the available literature [57–59], in agreement with our results. The increase in plant weight due to foliar sulfur fertilization was also documented. Perveen et al. [60] observed a significant increase in root and shoot biomass and root and shoot length of maize grown under salinity conditions due to the foliar application of different sulfur compounds. An increased barley yield after elemental sulfur application was described by Grzebisz and Przygocka-Cyna [61] in their long-term experiment. A positive effect of the foliar application of sulfur on canola pods formation and subsequent seed yield was demonstrated by Khalid et al. [62].

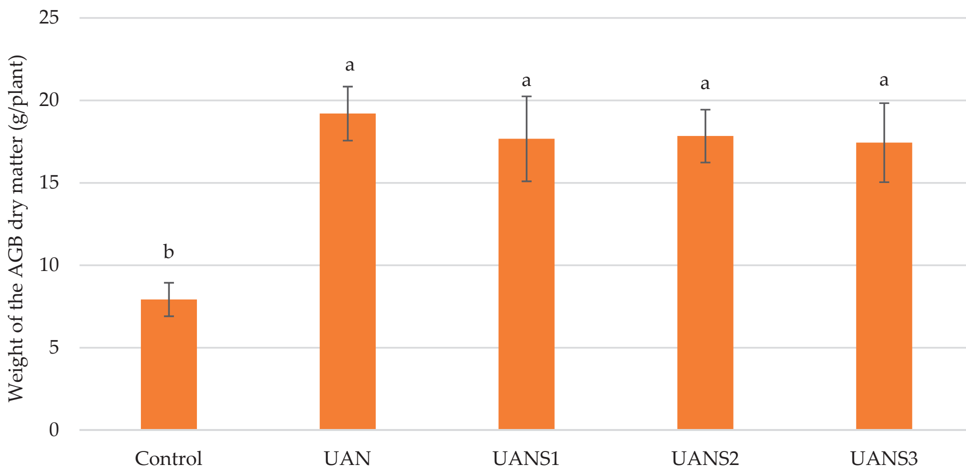

Figure 5. Weight of dry matter above-ground biomass of maize after the foliar application of fertilizer. The measurements were taken at the end of experiment (t3). The values represent the arithmetic mean (n = 8); the bars represent the standard deviation of the mean. There are no statistical differences between columns with the same letters (Fisher's LSD test, $p < 0.05$). AGB—above-ground biomass.

The UAN fertilizer application significantly increased the nitrogen content of maize leaves. The highest N content, 10.3 g/kg DM, was found in leaves after the application of UAN fertilizer alone (Table 2). There was no significant difference in plant N content among treatments fertilized with a mixture of UAN and elemental sulfur (UANS1-3), but the data showed a relative increase in N content with sulfur rate. An increased leaf N concentration following sulfur fertilization has also been described [31,63]. An increase in the nitrogen content of wheat grain, due to the foliar application of sulfur, was observed by Tea et al. [64] and Rossini et al. [65]. This effect could be due to a better assimilation of foliar-applied N and S compared to their soil-applied counterparts.

Table 2. Nutrient content and nutrient uptake by DM of AGB and the N:S ratio.

Treatment	Nitrogen		Sulfur		N:S Ratio
	g/kg DM	Rel. %	g/kg DM	Rel. %	
Control	7.7 ± 0.8 ^b	100.0	3.4 ± 0.6 ^a	100.0	2.3 ± 0.5 ^b
UAN	10.3 ± 1.8 ^a	134.0	2.8 ± 0.6 ^b	80.9	3.8 ± 0.4 ^a
UANS1	9.3 ± 0.6 ^a	121.6	3.6 ± 0.4 ^a	104.4	3.1 ± 0.8 ^ab
UANS2	9.7 ± 0.7 ^a	127.1	3.3 ± 1.3 ^a	97.1	3.4 ± 1.6 ^ab
UANS3	9.9 ± 0.5 ^a	129.7	3.3 ± 0.7 ^a	97.8	2.6 ± 0.4 ^ab

The values in the table represent the arithmetic mean (n = 8) ± SD (standard deviation). The same letters next to the numbers describe no statistically significant differences between the treatments (Fisher's LSD test, $p < 0.05$). DM—dry matter, AGB—above-ground biomass.

Sutar et al. [22] described the critical sulfur concentration in dry matter of maize leaves as 1.5 g/kg DM. The sulfur content in the ABG of maize plants ranged from 2.8 to 3.6 g/kg DM (Table 2). Its content in the ABG of plants grown on the treatments fertilized with a mixture of UAN and elemental sulfur (UANS1–3) was identical to that of unfertilized plants (Control). Only in the nitrogen-fertilized treatment (UAN) was the amount of sulfur significantly lowest (Table 2). This fact is not only related to the absence of sulfur in the fertilizer, but it can also be explained by the dilution of nutrients in the maize plant tissue that occurred as a result of the increase in DM weight of AGB on this treatment (Figure 5). Therefore, the nutrient uptake by the plant was calculated as a more appropriate parameter expressing the nutritional status of the plants (Figure 6). Nutrient uptake is the relationship between the DM weight of AGB and its nutrient content, expressed in g of nutrient per plant (g/plant). Logically, the highest nitrogen uptake was recorded in the UAN-fertilized treatment, i.e., the treatment with the highest applied nitrogen rate. Even though nitrogen uptake by plants was not significantly different among the treatments fertilized with UAN and elemental sulfur mixtures, plants fertilized with fertilizers containing a higher proportion of elemental sulfur (UANS2 and UANS3) showed a higher uptake of nitrogen by plant AGB. A positive significant interaction between nitrogen and sulfur uptake and utilization was confirmed.

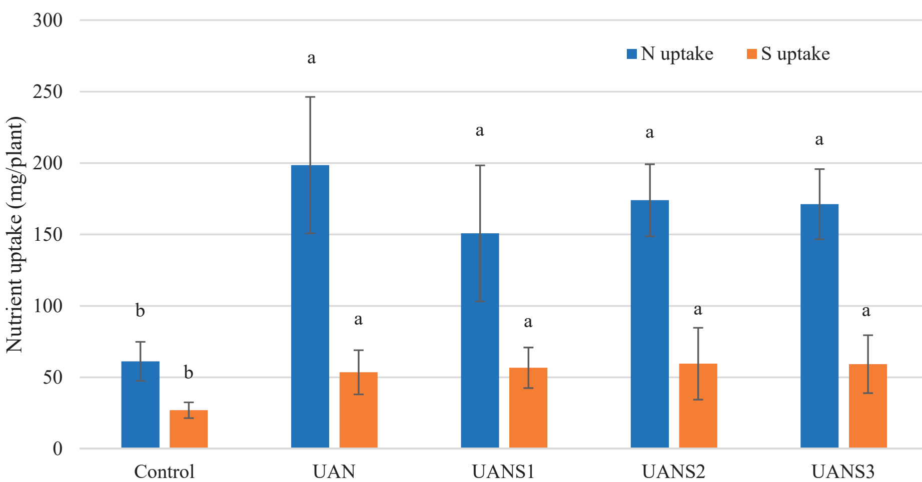

Figure 6. Nitrogen and sulfur uptake by above-ground plant biomass (mg/plant). The measurements were taken at the end of the experiment (t3). The values represent the arithmetic mean (n = 8); the bars represent the standard deviation of the mean. There are no statistical differences between columns with the same letters (Fisher's LSD test, $p < 0.05$).

The N:S ratio of the plant may also be an interesting indicator of nutritional status, as reported by some authors [31,66]. The principle behind this assessment is the fact that plants need a balanced amount of nitrogen and sulfur for proper amino acid synthesis. Therefore, nitrogen-to-sulfur ratios above a N:S ratio threshold indicate S deficiency [67]. A possible disadvantage of this assessment is the decreasing value of the N:S ratio during the growing season, as reported, for example, by Calvo et al. [68,69] or Scherer [70]. A 15-19:1 N:S ratio has been reported as a limiting ratio for cereals at the time of tillering [71], and an ideal N:S ratio for the optimum growth and development of maize is 15:1 [72]. The observed N:S ratio (Table 2) indicated that the sulfur contained in maize was not deficient in any of the fertilization treatments. From the ratios obtained, it is possible to observe the already described trend, where the highest ratio of nitrogen and sulfur was logically found on the treatment fertilized only with UAN fertilizer. In contrast to our study, significant changes in the N:S ratio after sulfur application were observed [73,74]. However, they agreed that an increase in the sulfur content of the plant does not necessarily predict increased yield. Sutradhar et al. [31] also confirmed the same conclusion.

The previously mentioned synergism between nitrogen and sulfur can be documented by crop nitrogen use efficiency. The nitrogen supplied by foliar nutrition from fertilizer applied without sulfur addition (UAN) was utilized by the plant at 30.5% (Table 3). A similar level of NUE_{Crop} was found on the treatment fertilized with the lowest sulfur fertilizer mixture (UANS1), whereas an increase in the proportion of sulfur in the fertilizer mixture increased nitrogen use efficiency. The relationship between nitrogen recovery from applied fertilizers and the dose of sulfur applied by the fertilizer mixture was statistically significant ($NUE_{Crop} = 22.9 + 0.171 \times$ sulfur dose, r = 0.709; $p = 0.002$).

Table 3. Crop nitrogen use efficiency.

Treatment	NUE_{Crop}	
	%	Rel. %
Control	-	-
UAN	30.5 ± 5.3^{b}	100.0
UANS1	29.9 ± 7.9^{b}	97.8
UANS2	50.1 ± 5.6^{b}	164.2
UANS3	73.4 ± 8.2^{a}	240.3

The values in the table represent the arithmetic mean ($n = 8$) ± SD (standard deviation). The same letters next to the numbers describe no statistically significant differences between the treatments (Fisher's LSD test, $p < 0.05$).

In agreement with our results, several studies showed that sulfur fertilization may increase NUE [75–77]. As sulfur is an essential constituent of enzymes involved in nitrogen metabolism [78], its deficiency may lead to ineffective utilization of the nitrogen content in plant [79,80]. An increase in nitrogen uptake by maize plants due to graded doses of foliar sulfur application was presented by Sarfaraz et al. [81].

3. Materials and Methods

3.1. Experimental Methodology, Plant Material, and Growth Conditions

The pot vegetation experiment was established in the vegetation hall of the Biotechnological house at Mendel University in Brno located at 49°21′03″ N and 16°61′38″ E. Mitscherlich pots (STOMA GmbH, Siegburg, Germany) were filled with 6.5 kg of air-dried and sieved soil (2 cm diameter sieve). Properties of the soil used in the pot experiment are shown in Table 4.

Table 4. Properties of soil used in pot experiment.

Soil Parameter	Value	Ref
pH ($CaCl_2$)	6.09	[82]
Soil oxidizable carbon (Cox)	0.80%	[83]
Clay	20%	[84]
Silt	27%	[82]
Sand	53%	[82]
Cation Exchange Capacity	164 mmol/kg	[82]
N total	0.19%	[82]
N-NH_4^+ (K_2SO_4)	1.48 mg/kg	[82]
N-NO_3^- (K_2SO_4)	17.2 mg/kg	[82]
S (water soluble)	8 mg/kg	[82]
P (Mehlich 3)	36.4 mg/kg	[82]
K (Mehlich 3)	400 mg/kg	[82]
Ca (Mehlich 3)	2720 mg/kg	[82]
Mg (Mehlich 3)	214 mg/kg	[82]

The maize (*Zea mays* L.), cultivar SY ORPHEUS (Syngenta Czech s.r.o., Prague, Czech Republic), was chosen for this study. Four seeds of maize were sown to a 4 cm depth in each pot. The number of plants in each pot was reduced to two plants per pot two weeks after the sowing.

The pot experiment was carried out under seminatural conditions in the outdoor vegetation hall under a rain shelter. The air temperature, air humidity, and solar radiation during the maize growing season are shown in Figure 7. After a cooler April (11.8 °C) and May (12.6 °C), a warming period occurred at the beginning of June (22.6 °C), which lasted until the end of the experiment (average air temperature in July was 19.6 °C). The relative air humidity fluctuated evenly between 40 and 90% during the experiment. Global solar radiation also fluctuated over time depending on weather conditions, with levels increasing slightly during the experiment (April: 16.9; July: 22.1 MJ/m^2). A controlled watering regime identical for all treatments (pots) was used in the experiment. Plants were watered to 70% of the maximum water holding capacity throughout the growing season. The pots were watered by hand with demineralized water on the soil surface.

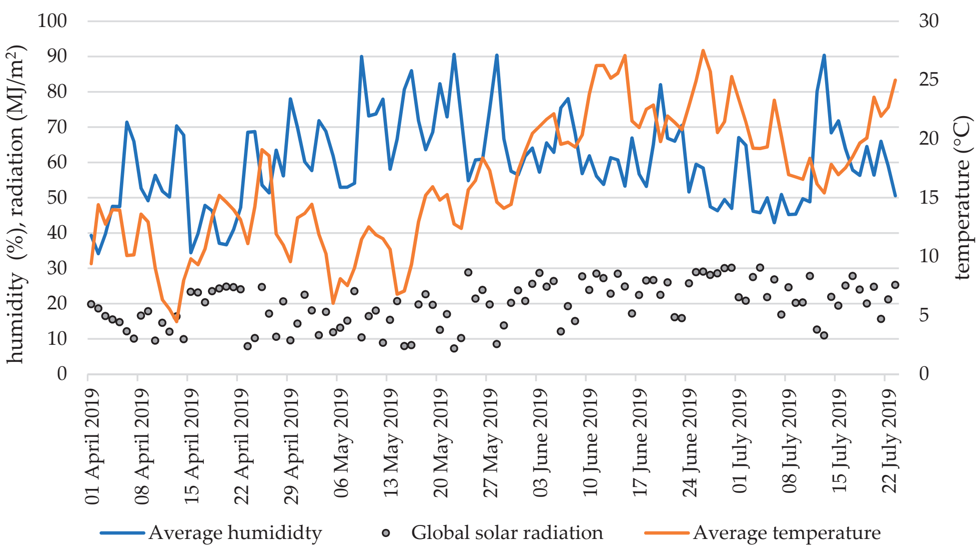

Figure 7. The average daily temperature (°C), relative humidity (%), and global solar radiation (MJ/m^2) in the vegetation hall during the experiment.

Liquid urea ammonium nitrate fertilizer (UAN; 30% total N–15% N-NH_2, 7.5% N-NO_3, 7.5% N-NH_4) was applied to maize plants in combination with waste elemental sulfur suspension (12% S^0 suspension) in the ratios shown in Table 5. The sulfur suspension was obtained by the desulfurization of biogas using the Thiopaq® scrubber (Paques, Balk, The Netherlands), which works by washing the raw biogas with a slightly alkaline solution (pH 8–9) and the subsequent biological oxidation of sulfides to elemental sulfur. The elemental sulfur particle size in the suspension was less than 60 µm (96.9% of the particles). Each of the treatments was established in eight replicates (pots). The pots were placed randomly in the vegetation hall under the rain shelter.

The foliar application was carried out on the plant development stage of the 4th true leaf unfolded. The application of 3 mL of fertilizer mixture per pot of each treatment was used. Fertilizers were evenly applied using a pressurized hand pump sprayer (DPZ 1500, ProGlass, Weilheim an der Teck, Germany). The mixture of the waste elemental sulfur with the UAN fertilizer was mixed prior to application to ensure that the elemental sulfur was evenly distributed in the fertilizer mixture and applied uniformly.

During the maize vegetation, chlorophyll content (N-tester value) and chlorophyll fluorescence parameters were evaluated. The measurements were performed 7 (t1) and

21 days (t2) after the foliar application. The weight of dry matter (DM) of maize plant AGB, the content and ratio of nutrients (N and S) in maize plant AGB, and their uptake by plants were determined 35 days after the foliar application of fertilizer mixtures (t3). The schedule of the experiment is shown in Table 6.

Table 5. Experimental treatments of foliar application in pot experiment.

Treatment	Proportion of Fertilizer in the UANS Mixture		Nutrient Content in the UANS Mixture (Weight %)	
	UAN	S Suspension	N	S
Control	0	0	0	0
UAN	100%	0	30	0
UANS1	66%	33%	20	4
UANS2	50%	50%	15	6
UANS3	33%	66%	10	8

Table 6. Timetable of the experiment.

Term		Growth Stages of Maize	Operation
1 April 2019		seed	Maize sowing
17 June 2019		4th true leaf	Foliar application of fertilizer mixtures
24 June 2019	t1	5th true leaf	Measurement of chlorophyll content and fluorescence parameters
8 July 2019	t2	6th true leaf	Measurement of chlorophyll content and fluorescence parameters
22 July 2019	t3	7th true leaf	Measurement of AGB harvest, DM weight, content and uptake of nutrient, N:S ratio

3.2. Determination of Plant Growth and Development Parameters

3.2.1. Chlorophyll Content in Plant Leaf (N-Tester Value)

The chlorophyll content of maize leaves was measured using a Yara N-tester (Yara International ASA, Oslo, Norway). The chlorophyll content was expressed as "N-tester value." Measurement was performed at a wavelength range of 650–940 nm [85]. Eight plants were assessed in each treatment in both terms. The measurement of chlorophyll content was performed on the 5th (t1) and 6th true leaves (t2), and the value of the chlorophyll content of each plant was the mean of 60 measurements.

3.2.2. Chlorophyll Fluorescence Parameters

To determine the photochemical efficiency of photosystem II, selected fluorescence parameters of chlorophyll were measured in maize plant. The tested parameters were measured with the PAR-FluorPen FP 110-LM/S (Photon Systems Instruments, Drásov, Czech Republic) and evaluated using the FluorPen 1.1 software [86]. Measurements of fluorescence parameters were carried out on identical leaves (5th and 6th true leaves) at identical terms (t1 and t2) as for chlorophyll content determination. Maize leaves were dark-adapted for 25 min before measurement. The protocol for measuring the fluorescence parameters of chlorophyll is shown in Table 7.

The variable fluorescence of the dark-adapted leaves (F_v), quantum yield of photosystem II (Φ_{PSII}), and chlorophyll fluorescence decrease ratio (R_{Fd}) were determined (Table 8).

Table 7. Measurement protocol of the chlorophyll fluorescence parameters.

Chlorophyll Fluorescence Parameters	Pulse Type	Light Intensity ($\mu mol/m^2/s$)	Phase	Duration (s)	1st Pulse (s)	Pulse Interval (s)
Φ_{PSII}, F_v	Saturation	2400	-	1 pulse		
R_{Fd}	Flash	900	L	60	0.2	1
			DR	88	1	1
	Saturation	2400	L	60	7	12
			DR	88	11	26
	Actinic	300	L	60	-	-

The measured at wavelength (λ) of 454 nm, L—light, DR—dark recovery, Φ_{PSII}—quantum yield of photosystem II, R_{Fd}—chlorophyll fluorescence decrease ratio, F_v—variable fluorescence of the dark-adapted leaves.

Table 8. The photochemical quenching parameters.

Chlorophyll Fluorescence Parameters		Ref.
F_v	$F_m - F_0$	[87]
Φ_{PSII}	$F_m - F_0 / F_m$	[88]
R_{Fd}	F_d / F_s	[89]

F_0—minimal fluorescence from the dark-adapted leaves, Φ_{PSII}—quantum yield of photosystem II, R_{Fd}—chlorophyll fluorescence decrease ratio, F_m—maximal fluorescence from the dark-adapted leaves; F_d—fluorescence decrease from F_m to F_s; F_s—steady-state chlorophyll fluorescence.

3.2.3. Determination of Weight Biomass, Nutrient Contents and Uptake, and Nitrogen Use Efficiency

The ABG of maize plants was harvested on 22 July 2019 (t3). The ABG was then oven-dried at 60 °C for the first two hours. The temperature was then reduced to 45 °C where the samples were kept for 72 h. The dry weight of ABG was determined using a laboratory-scale PCB Kern (KERN & Sohn GmbH, Balingen, Germany). Then, the dried ABG was crushed and homogenized by the grinder Grindomix GM200 (Retsch GmbH, Haan, Germany). The HNO_3/H_2O_2 [90] digestion of biomass was achieved using a microwave digestion system in ETHOS 1 (Milestone Srl, Sorisole, Italy). Subsequently, the nutrient content (Table 9) and nutrient ratio were determined, and crop nitrogen use efficiency (NUE_{Crop}) was calculated by the relationship NUE_{Crop} = N yield/N input · 100 (N yield = nitrogen uptake by plants (mg/pot); N input = nitrogen applied by foliar fertilizers (mg/pot)) [91]. In the calculation of NUE_{Crop}, the nitrogen uptake by plants grown on the fertilized treatments (N yield) was subtracted from the nitrogen uptake observed on the control treatment (this amount of nitrogen characterized the natural soil supply).

Table 9. The methods for the determination of nutrients in maize AGB.

Nutrient	Method Used	Device Used	Ref.
N	Kjeldal method	Kjeltec 2300 device (Foss Analytical, Hillerød, Denmark)	[92]
S	Optical emission spectrometry	ICP–OES (Spectro, Kleve, Germany)	[93]

3.3. Statistical Data Analysis

The effect of the foliar application of fertilizer mixtures on the plant growth and development parameters was statistically analyzed by the STATISTICA 12 program (TIBCO Software, San Jose, CA, USA) [94]. The effect of the foliar application on the N-tester value, chlorophyll fluorescence parameters, dry weight of ABG, and nutrient content ABG of maize was analyzed separately for each treatment of the experiment. The normality was checked using the Shapiro–Wilk test, and the homogeneity was verified by the Levene test at $p \leq 0.05$. The effect of fertilization was analyzed using ANOVA. Fisher's LSD test ($p \leq 0.05$) was used to determine any statistically significant differences between the means of treatments.

4. Conclusions

Nitrogen plays an important role in maize nutrition, contributes to chlorophyll formation, and significantly influences the photosynthetic activity of plants. The result of the vegetation experiment showed that the efficiency of mineral nitrogen fertilization can be increased by the foliar application of liquid fertilizers with sulfur addition. The optimal adjustment of the ratio of applied nutrients (N and S) improves the nutritional status of the plants and allows the reduction in their doses while minimizing the environmental risks associated with fertilization. The foliar application of UAN fertilizer in combination with elemental sulfur from biogas production in a 1:1 ratio seems to be a sensible way to optimize the nutritional status of maize, both for the economics of biogas purification, when the waste sulfur is reused as a fertilizer, and for environmental reasons. However, verification of the results obtained from the pot experiment in field trials will be necessary.

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Magnesium Fertilization Increases Nitrogen Use Efficiency in Winter Wheat (*Triticum aestivum* L.)

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Abstract: Wheat fertilized with Mg, regardless of the method of application, increases nitrogen fertilizer (N_f) efficiency. This hypothesis was tested in 2013, 2014, and 2015. A two-factorial experiment with three doses of Mg (i.e., 0, 25, and 50 kg ha^{-1}) and two stages of Mg foliar fertilization (without; BBCH 30; 49/50; 30 + 49/50) was carried out. Foliar vs. in-soil Mg fertilization resulted in a comparable grain yield increase (0.5–0.6 t ha^{-1}). The interaction of both fertilization systems increased the yield by 0.85–0.9 t ha^{-1}. The booting/heading phase was optimal for foliar fertilization. Mg accumulation by wheat fertilized with Mg increased by 17% compared to the NPK plot. The recovery of foliar Mg was multiple in relation to its dose. The recovery of the in-soil Mg applied ranged from 10 to 40%. The increase in yield resulted from the effective use of N taken up by wheat. In 2014 and 2015, this amount was 21–25 kg N ha^{-1}. The increase in yield resulted from the extended transfer of N from vegetative wheat parts to grain. Mg applied to wheat, irrespective of the method, increased the efficiency of the N taken up by the crop. Mg fertilization resulted in higher N_f productivity, as indicated by the increased nitrogen apparent efficiency indices.

Keywords: in-soil Mg application; foliar Mg fertilization; Mg uptake; N uptake; nutrient use efficiency indices

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1. Introduction

Wheat is a basic source of staple food for the world's human population. Currently, the largest producers are China, India, the Russian Federation, the USA, Canada, France, and Ukraine. The mean yields in these countries in 2018–2021 were 5.6 ± 0.17, 3.4 ± 0.12, 2.8 ± 0.15, 3.9 ± 0.23, 3.4 ± 0.01, 7.1 ± 0.58, and 3.3 ± 0.14, respectively [1]. The yield variability, as shown by the coefficient of variation (CV), in leading producers such as Canada, China, and India was 3.5%, 3.0%, and 2.5%, respectively. This low CV indicates an extensive system of wheat production. Therefore, in these countries, the yield gap (YG), as a measure of the ineffectiveness of the applied means of production, is low [2]. For Germany, the average yield in 2018–2020 was 7.3 ± 0.58 t ha^{-1}, compared to 4.5 ± 0.65 t ha^{-1} for Poland. The CV for Germany was 7.9% and for Poland 14.4%.

A realistic determination of the maximum attainable yield, as the basis for calculating the YG, requires local data that reflect both the short-term variability in weather conditions and the variability in management factors affecting the actual yield. The order of these factors for wheat in Poland, in descending order, is nitrogen (N), fore-crop, soil class, available potassium content, crop protection level—fungicide and foliar fertilization, and available phosphorus content [3]. Another method for the calculation of the YG, which is, in fact, simpler to use, is the concept of the nitrogen gap [4,5]. Both methods allow for the discrimination of factors, which is critical for the maximum wheat yield in a well-defined geographical area [6].

The nutrient requirements of wheat compared to other cereals is high, including, firstly, N, P, and K [7]. The yield-forming function of N is widely discussed in both science and agricultural practice. Some of the most important data on the impact of N on yield in wheat concern the *critical window* that extends from the beginning of stem elongation to flowering. N supply to wheat during this period is critical for both the number of ears and the number of grains per ear. Both yield components determine the aggregate component of the yield, i.e., the number of grains per unit area (grain density (GD) [8,9].

New, high-yielding wheat varieties developed to exploit their yield potential, require, first, the development of efficient technologies aimed at the effective use of nitrogen fertilizer (N_f). The price of N_f increased many times in 2021 and 2002 [10]. The 4R Stewardship approach, the keywords of which are right source, right rate, right time, and right place, is actually limited by N_f [11].

Magnesium (Mg) cannot be treated as a *forgotten* nutrient, especially in modern, intensive, and effective agriculture [12,13]. The physiological and yield-forming functions of Mg are well known [14–16]. The increase in the yield of cereals in response to the application of Mg ranges from 5 to 10% [17]. The Mg content in the flag leaf of winter wheat at the onset of plant flowering can be used to forecast the yield [18]. The amount of Mg accumulated in crop plants is relatively low compared even to phosphorus [14]. In agricultural practice, farmers use two basic Mg fertilization systems, differing in the method of Mg fertilizer application to crops. The first, a classic method, is the in-soil application of Mg fertilizer using lime (Mg oxide or Mg carbonate) for acidic soils and magnesium sulfate (Kieserite) for soils with an optimum pH [19]. Mg availability to plants is not a problem, provided the content of its available form in the soil is in the medium class, at least [19]. The second method of Mg supply to crop plants is foliar fertilization. Magnesium is widely used in this way throughout the world [20,21]. The key challenge for farmers using soluble Mg fertilizers (i.e., sulfate or chloride) is to determine the (i) rate, (ii) methods, and (iii) time—the plant growth stage of fertilizer application. A well-developed Mg fertilization system should be oriented toward an increase in the efficiency of N_f.

The interaction between Mg and N occurs at all levels of a plant's organization. The importance of N for a plant's growth and yield results from its presence in key biological molecules such as chlorophyll and the ribulose bisphosphate carboxylase–oxygenase enzyme, simply called Rubisco (RuBP) [22]. The latter is a key N-dependent enzyme, decisive in the survival of life on Earth. Its key function is to capture and then fixate the CO_2 molecule, which is the basic substrate for the production of elementary sugar compounds [23,24]. The key function of Mg is to maintain Rubisco activation, which results in the stabilization of the net photosynthetic rate, as was demonstrated for wheat by Shao et al. [25]. Crop plants well supplied with Mg since the beginning of their growth increases N uptake, resulting in an increase in its unit productivity [26,27]. For example, Mg concentration in maize leaves during the grain filling period is the critical factor affecting the grain yield of this crop. The adequate nutrition of plants with Mg increases N productivity, in turn decreasing the required N_f dose [28,29].

Two main questions remain open. The first, and most important, concerns the relationship between N and Mg from the point of view of the impact of Mg on the management of N by arable crops. The N/Mg ratio during the early growth of potato tubers or sugar beets is crucial for the final yield of both crops [20,27,30,31]. The second, more minor one concerns the efficiency of the applied Mg, depending on the method of its application. The main objective of this study was to evaluate (i) the effectiveness of applied Mg depending on the fertilization method and (ii) the relationship between Mg and N uptake by wheat and its use efficiency. A secondary objective was to evaluate Mg fertilization systems including soil and foliar fertilization of wheat fertilized with an optimum dose of N_f.

2. Results

2.1. Magnesium Fertilization Systems and Yield Increment

The yield gain due to the application of Mg to winter wheat was the result of the interaction between the soil and the foliar treatment (Figure 1). Detailed information on the grain yield and the elements of the yield structure can be found in an article by Grzebisz and

Potarzycki [32]. In all years of the study, Mg application, regardless of the Mg fertilization system, increased the grain yield. The recorded increase ranged from approximately 0.58 to 0.74 t ha^{-1}. The lower yields were due to unfavorable weather conditions during the spring growing season (2013 and 2015). Nevertheless, the effect of the Mg fertilization system (Mg-FS) on the yield gain in the consecutive years of the study was highly stable (Figure A1). A strong interaction between the Mg and FSs was observed (Figure 1). The increase in yield due to the soil-applied Mg (Mgs) in comparison with the absolute Mg control (the plot treated only with NPK) amounted to approximately 0.52 t ha^{-1}. The effect of a single stage of Mg foliar treatment (Mgf), regardless of the growth stage of wheat, was 0.57 t ha^{-1}. A double stage of Mg foliar application (at BBCH 30 and repeated at BBCH 49/50) resulted in a yield increase of 0.74 t ha^{-1}. The effect of the interaction of both Mg and FSs with the yield gain was dependent on the dose of Mgs. The increase in yield was 0.77 t ha^{-1} for the plot fertilized with 25 kg Mg ha^{-1}, but it provided Mg foliar application at BBCH 30. The same level of increase in the yield was recorded on the plot with 50 kg Mg ha^{-1}, regardless of the growth stage of wheat treated with Mg. In-soil and double, two-phase foliar feeding of wheat with Mg resulted in a yield gain of 0.92 t ha^{-1}.

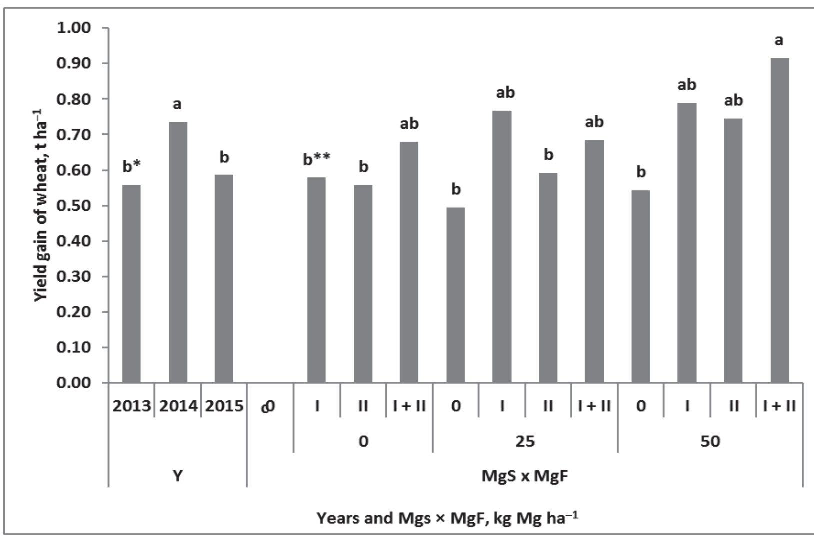

Figure 1. The net increase in the yield of winter wheat in response to the interaction between Mg fertilization systems. a, b. Similar letters mean a lack of significant differences using Tukey's test. Mgs and Mgf—soil and foliar Mg fertilization systems, respectively. * Doses of applied Mg, kg ha^{-1}; ** stages of Mg. Wheat stages of Mg foliar fertilization: I—BBCH 30; II—BBCH 49/50.

2.2. Magnesium Accumulation and Indices of Efficiency

Magnesium accumulation in wheat at maturity was significantly dependent on the course of the weather during the growing season (Table 1). The effect of the Mg fertilization system was not significant for grain, but it was significant for wheat residues (straw + chaffs). The total Mg accumulated by wheat at harvest resulted from the interaction between the Mg fertilization systems and years (Figure 2). In 2013, the effect of the Mgs was visible only on the Mgs50 plot. The effect of Mgf was the strongest on the Mgs25 main plot. Plants foliar fertilized at the BBCH 49/50 stage increased Mg accumulation by 2 kg ha^{-1} compared to its uptake on the Mgs control plot. In 2014, the uptake of Mg was, in general, higher than in 2013 and 2015. In the Mgs control plot, the double-stage foliar fertilization of wheat increased the uptake of Mg by 2.0 kg ha^{-1} compared to the Mg absolute control. The effect of the Mgs was only slightly stronger on the Mgs50 than on the Mgs25 main plot. The interaction between both fertilization systems was the strongest on the Mgs50 plot and the double-stage Mgf treatment. Mg extra uptake was 2.9 and 2.5 kg ha^{-1} higher compared to the Mg absolute control and the Mgs50 control, respectively. In 2015, the average Mg accumulation by wheat was 30% and 21% lower compared to 2014 and 2013, respectively. The positive effects of Mgf on the uptake of Mg were observed only on the Mgs plots. The strongest increase in Mg accumulation by wheat in response to Mgf was observed on the Mgs50 plot, reaching the maximum for the double-foliar-treated plants (BBCH 30 + 49/50). The greatest amount of Mg taken up by wheat during the growing season (i.e., slightly above two-thirds) was accumulated in grain as indicated by the Mg harvest index (Mg–HI).

Only two indices of Mg productivity (i.e., the Mg unit accumulation in grain (MgUA–G) and the total Mg unit accumulation (MgUA–T) of the nine), showed a significant but negative relationship with the yield (Table A1). Four of the five indices (Mg–HI, MgUA–G, MgUA–T, and Mg unit productivity–grain (MgUP–G)) showed year-to-year variability, but their response to the interaction Y × Mgs × Mgf was not significant. The only significant dependence was noted for the Mg total unit productivity (MgUP–T). However, this index was negatively correlated with the total Mg accumulation (r = –0.78 ***). Moreover, its highest values were recorded in the dry year of 2015, when they were 46% and 28% higher compared to 2013 and 2014, respectively.

Four classic efficiency indices of the applied nutrients (PFP–Mg, Mg–AE, Mg–R, and Mg–PhE) responded significantly to all studied factors including Y × Mgs × Mgf (Table 1). However, none of them showed a significant relationship with the yield (Table A1). All these indices displayed a steady yearly trend, clearly highlighting much higher values in 2014, the year with the highest yield. A significant effect of the experimental treatments on these indices resulted from the increase in the applied doses of Mg from 2.4 to 56.4 kg ha^{-1}. This trend can be described by a power regression model. The general trend in the data obtained is presented in detail for Mg recovery (Mg–R) (Figure A2). Its values, regardless of the year of the study, were the highest for the plots with only foliar-applied Mg. The recovery of the applied Mg for these treatments exceeded 100%. The differences between years were the clearest for the Mgf dose of 2.4 kg ha^{-1}, reaching 383% in 2014, 300% in 2013, and 260% in 2015. The developed regression models clearly showed that the yearly pattern, observed for the lowest dose of Mg, was maintained with an increase in the Mg dose (Figure A3). The impact of the Mgs × Mgf interaction on the Mg-R values was observed only on the main Mgs25 plot.

2.3. Nitrogen Accumulation and Indices of Efficiency

The total amount of N accumulated in winter wheat at maturity (TN) significantly depended on the experimental factors. In fact, they influenced the accumulation of N, but the interaction with years was only noted for wheat residues (Table 2). The amount of N in grain (N_aG) was the result of independent interactions of the year with Mg fertilization systems (Y × Mgs and Y × Mgf). The first were revealed in 2014 and 2015. In both years, the Mgs caused a significant increase in N_aG compared with the Mg absolute control. A significant effect of Mgs50 was only observed in the dry year of 2015 (Figure 3). The effect of the Mgf was most evident in 2013 (Figure 4). In 2014, it was only slightly marked at BBCH 30. In 2015, it was much more visible, but a significant difference was only noted between the Mgs control and the plot with double Mg foliar fertilization (BBCH 30 + 49/50). The same principles were noted for the nitrogen harvest index (NHI). The values of this index were very high, approaching almost 90% in 2014.

Table 1. Magnesium uptake by winter wheat at maturity and indices of magnesium use efficiency.

Factor	Level of Factor	Mg_aG	Mg_aCR	MgaT	MgHI	MgUA–G	MgUA–T	MgUP–G	MgUP–T	PFP–Mg	MgAE	Mg–R	Mg–PhE
		kg ha^{-1}			%	kg Mg t^{-1}		kg Grain kg^{-1} Mg			t Grain kg^{-1} Mgf	%	t Grain kg^{-1} Mg_aT
Year (Y)	2013	11.1 b	5.3 b	16.4 b	67.9 a	1.31 a	1.93 a	766.2 c	519.9 c	731.8 c	0.36 b	62.6 b	0.66 b
	2014	12.6 a	5.8 a	18.4 a	68.4 a	1.15 b	1.69 b	871.6 b	595.4 b	946.8 a	0.44 a	81.6 a	1.05 a
	2015	8.3 c	4.6 c	12.9 c	64.8 b	0.86 c	1.33 c	1178.8 a	760.5 a	843.3 b	0.34 b	54.4 c	0.56 b
p		***	***	***	***	***	***	***	***	***	***	***	***
Mg in-soil	0	10.6	5.1 a	15.6 b	67.4	1.11	1.65	921.5	620.1	1990.1 a	0.91 a	156.2 a	2.21 a
Mgs	25	10.7	5.1 a	15.8 ab	67.3	1.10	1.63	950.7	634.6	346.8 b	0.16 b	27.2 b	0.04 b
	50	10.8	5.5 a	16.3 a	66.3	1.11	1.67	944.3	621.2	185.0 c	0.09 c	15.2 c	0.01 c
p		n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	***	***
Mg foliar	0	10.4	4.8 b	15.2 b	68.3 a	1.11	1.63	929.0	633.2	191.9 d	0.09 d	14.0 d	0.02 d
(Mgs)	I	10.6	5.1 ab	15.7 ab	67.1 ab	1.09	1.62	959.9	638.5	1524.4 a	0.69 a	118.1 a	1.96 a
	II	10.9	5.3 ab	16.1 a	67.1 ab	1.12	1.67	919.5	614.2	975.2 b	0.44 b	79.7 b	0.75 b
	I + II	10.8	5.6 a	16.4 a	65.7 b	1.10	1.68	947.0	615.3	671.2 c	0.31 c	53.0 c	0.30 c
p		n.s.	***	***	*	n.s.	n.s.	n.s.	n.s.	***	***	***	***
Source of Variation for Interaction													
Y × Mgs		*	n.s.	*	*	*	n.s.	*	*	***	***	***	***
Y × Mgf		n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	***	***	**	***
Mgs × Mgf		n.s.	**	**	*	n.s.	n.s.	n.s.	***	***	***	***	***
Y × Mgs × Mgf		n.s.	*	*	n.s.	n.s.	n.s.	n.s.	**	***	***	***	***

a, b, c, d Similar letters mean a lack of significant differences using Tukey's test; ***, **, and * indicate significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; 30% n.s.—not significant. [1] I—BBCH 30; II—BBCH 49/50. Mgf—Mg fertilizer doses; Mg_aG, Mg_aCR, and Mg_aT—magnesium accumulation: grain, crop residues, and total, respectively; MgHI—magnesium harvest index; MgUA–G and MgUA–T—magnesium accumulation: grain and total, respectively; MgUP–G and MgUP–T—magnesium unit productivity: grain and total, respectively; PFP–Mg—partial factor productivity of fertilizer magnesium; MgAE—magnesium apparent efficiency; Mg–R—magnesium recovery; Mg–PhE—magnesium physiological efficiency.

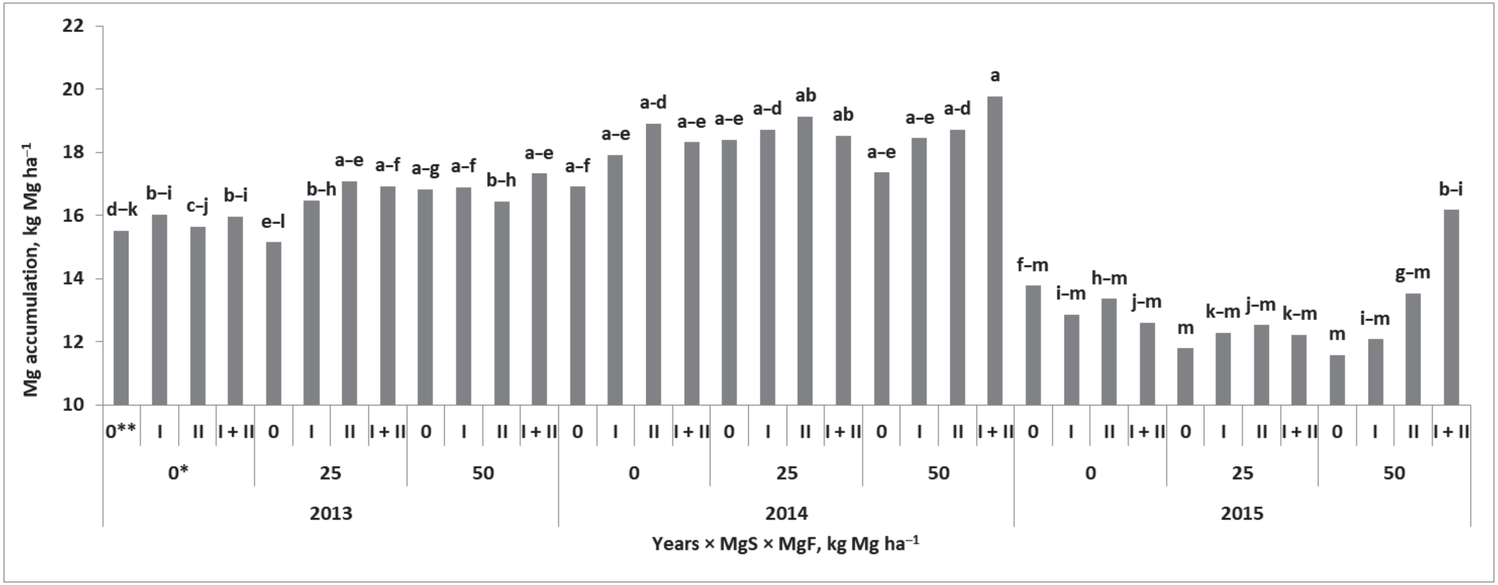

Figure 2. Magnesium accumulation by winter wheat in response to Mg fertilization systems and years. a, b, c, d, e, f, g, h, i, j, k, l, m Similar letters mean a lack of significance; differences using Tukey's test. Mgs and Mgf—soil and foliar Mg fertilization systems, respectively. * Doses of applied Mg, kg ha^{-1}; ** stages of Mg foliar fertilization to wheat: I—BBCH 30; II—BBCH 49/50.

Table 2. Nitrogen uptake by winter wheat at maturity and indices of nitrogen use efficiency.

Factor	Level of Factor	N_aG	N_aCR	TN	NHI	NUA–G	NUA–T	NUP–G	NUP–T	PFP–N	NAE	N–R	N–PhE
		kg N ha^{-1}			%	kg N t^{-1}		kg Grain kg^{-1} N			Grain kg^{-1} N_f	%	Grain kg^{-1} TN
Year (Y)	2013	185.0 c	46.8 b	231.8 c	79.8 c	21.9 b	27.4 b	45.8 b	36.5 b	44.5	22.1 b	78.9 b	28.0 b
	2014	224.5 b	30.3 c	254.8 b	88.1 a	20.5 c	23.3 c	49.0 a	43.1 a	57.5	27.1 a	81.5 b	33.6 a
	2015	265.0 a	59.0 a	324.0 a	81.8 b	27.3 a	33.4 a	36.7 c	30.0 c	51.0	20.7 c	107.2 a	19.4 c
p		***	***	***	***	***	***	***	***	***	***	***	***
Mg in-soil Mgs	0	214.5 b	45.1	259.6 b	82.6	22.6 b	27.5 b	45.1 a	37.4 a	50.2 b	22.4 b	83.6 b	27.8 a
	25	228.8 a	44.9	273.7 a	83.6	23.6 a	28.3 a	43.1 b	36.1 b	51.1 a	23.4 ab	91.0 a	26.4 a
	50	231.2 a	46.0	277.2 a	83.5	23.5 a	28.3 a	43.2 b	36.2 b	51.7 a	24.0 a	92.9 a	26.7 a
p		***	n.s.	***	n.s.	***	**	***	***	***	**	***	n.s.
Mg foliar (Mgf)	0	217.1 b	43.6	260.6 b	83.2	23.0	27.8	44.1	36.7	49.6 b	21.9 b	84.2 b	26.7
	I	228.6 a	45.5	274.2 a	83.5	23.4	28.1	43.4	36.3	51.5 a	23.8 a	91.3 a	26.8
	II	222.1 ab	46.1	268.1 ab	82.9	23.0	27.9	44.6	37.1	51.1 a	23.4 a	88.1 ab	27.8
	I + II	231.5 a	46.2	277.7 a	83.4	23.6	28.4	43.2	36.1	51.8 a	24.1 a	93.2 a	26.7
p		***	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	***	n.s.
Source of Variation for Interaction													
Y × Mgs		**	**	*	**	**	n.s.	***	**	n.s.	n.s.	n.s.	n.s.
Y × Mgf		*	*	n.s.	*	***	*	***	**	n.s.	n.s.	n.s.	*
Mgs × Mgf		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	*	n.s.	n.s.	n.s.
Y × Mgs × Mgf		n.s.	*	n.s.	n.s.	*	n.s.	*	*	n.s.	n.s.	n.s.	n.s.

a, b, c Similar letters mean a lack of significant differences using Tukey's test. ***, **, and * indicate significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; n.s. = not significant. I—BBCH 30; II—BBCH 49/50. N_f–nitrogen dose; N_aG, N_aCR, and N_aT—nitrogen accumulation: grain, crop residues, and total, respectively; NHI—nitrogen harvest index; NUA–G, NUA–T—nitrogen accumulation: grain and total, respectively; NUP–G and NUP–T—nitrogen unit productivity: grain and total, respectively; PFP–N—partial factor productivity of fertilizer nitrogen; NAE—nitrogen apparent efficiency; N–R—nitrogen recovery; N–PhE—nitrogen physiological efficiency.

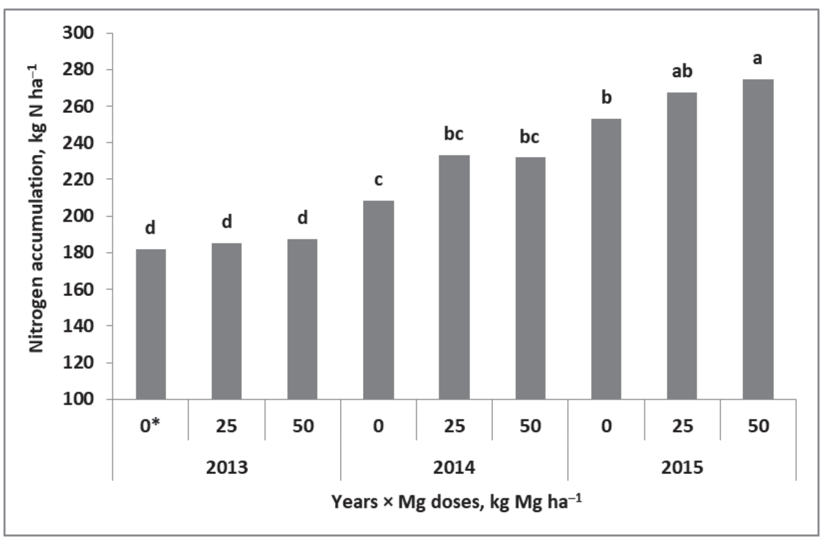

Figure 3. Effect of soil-applied magnesium to winter wheat on nitrogen accumulation in grain. a, b, c, d Similar letters mean a lack of significant differences using Tukey's test. Mgs—soil Mg fertilization system. * Doses of applied Mg, kg ha^{-1}.

Of the nine nitrogen use efficiency (NUE) indices studied, only three (i.e., nitrogen unit accumulation in grain (NUA–G), nitrogen unit productivity for grain (NUP–G), and total nitrogen unit productivity (NUP–T)) responded significantly to the Y × Mgs × Mgf interaction. The NUP–G was ultimately the most sensitive index (Figure 5). In 2013, its values showed no response to experimental factors, oscillating around 46 ± 0.8 kg grain kg^{-1} Na. In 2015, the NUP–G was significantly lower, on average, reaching only 37 ± 1.5. In 2014, the highest N productivity was found for the Mgs control. The highest NUP–G of 50 kg grain kg^{-1} Na was recorded on the plot with Mgf at the BBCH 49/50 stage. On plots with soil-applied Mg, the NUP-G indices were slightly lower than for the Mgs control but at the same time significantly responded to the Mgf treatments. Despite that, the NUP–G did not show a significant impact on the yield (Table A2). Moreover, it was negatively correlated with Na–G, Na–CR, TN, and, as a rule, with NUA–G and NUA–T, and also with N–R. At the same time, this particular index was significantly and positively correlated with N-PhE (r = 0.98 ***).

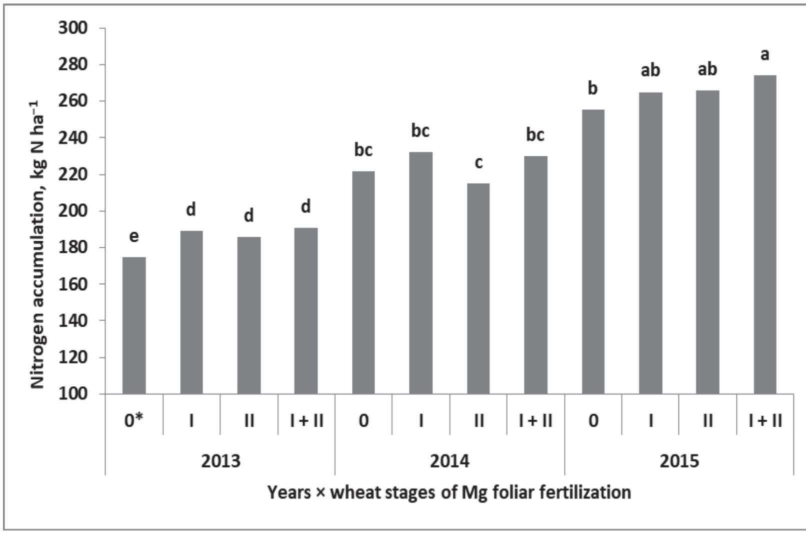

Figure 4. Effect of foliar fertilization of winter wheat with magnesium on nitrogen accumulation in grain. a, b, c, d, e Similar letters mean a lack of significant differences using Tukey's test. Mgf—foliar Mg fertilization system. * Stages of Mg foliar fertilization to wheat: I—BBCH 30; II—BBCH 49/50.

The classic NUE index, the partial factor productivity of fertilizer N (PFP–N_f), responded to the Mgs × Mgf interaction, and the nitrogen physiological efficiency (N-PhE) responded to the Y × Mgf interaction. The application of Mg significantly increased the productivity of N_f. The differences between the Mg absolute control plot and plots fertilized with Mg were significant. The strongest increase in PFP-N_f was recorded for Mgs50, which provided double-foliar fertilization with Mg. The highest variability in N–PhE indices was recorded in 2013 (Figure 6). Nitrogen utilization by wheat increased in response to Mg foliar fertilization, peaking when applied at BBCH 49/50. In the remaining two years, N–PhE showed significantly lower variability in response to Mgf, especially on plots with double Mg treatments. Two other NUE indices (i.e., nitrogen apparent efficiency (NAE) and nitrogen recovery (N–R)) responded significantly to the studied factors but did not interact with each other. The NAE indices were highly sensitive to the weather, having the highest values in 2013 (31% higher than in the dry year of 2015). A reverse trend was recorded for N–R, which was high in all years but exceeded 100% in 2015. The soil-applied Mg increased the values of both indices. The same effect was observed with Mg foliar application.

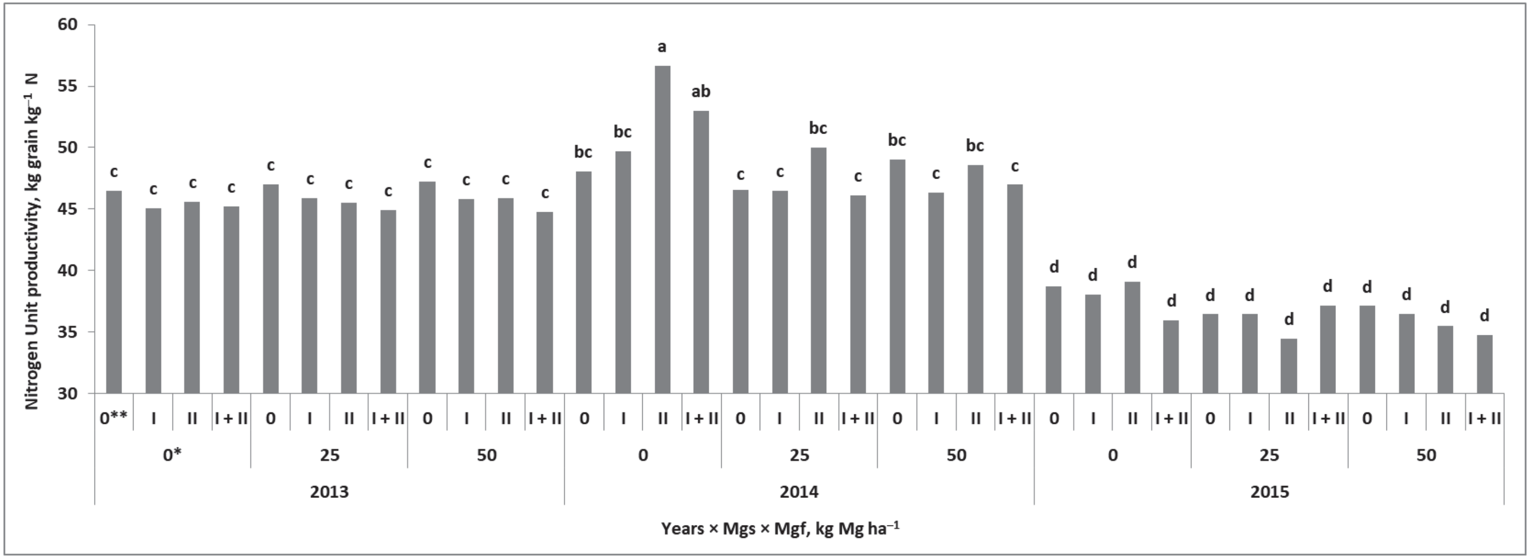

Figure 5. The nitrogen unit productivity of grain in response to the interaction of Mg fertilization systems and years. a, b, c, d Similar letters mean a lack of significant differences using Tukey's test. Mgs and Mgf—soil and foliar Mg fertilization system, respectively. * Doses of applied Mg, kg ha^{-1}; ** stages of Mg foliar fertilization to wheat: IBBCH—30; II—BBCH 49/50.

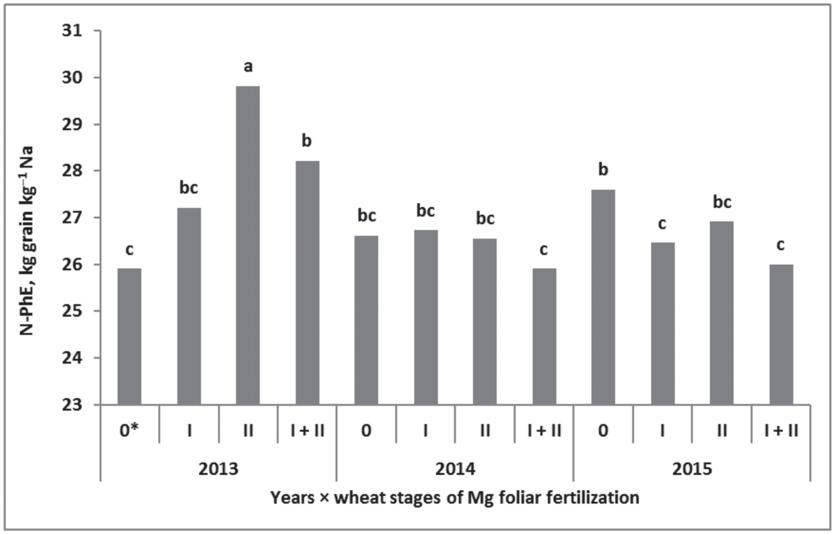

Figure 6. Effect of winter wheat foliar fertilization with magnesium on nitrogen physiological efficiency. a, b, c Similar letters mean a lack of significant differences using Tukey's test. Mgf—foliar Mg fertilization system. * Stages of Mg foliar fertilization to wheat: I—BBCH30; II—BBCH 49/50.

The wheat yield showed the highest correlation with N–HI and then with NAE (Table A2). The yield formula based on NHI, despite a statistically proven linear model, can be represented as a quadratic function:

$$\text{GY} = 0.26\text{NHI} - 11.54 \text{ for } n = 36, R^2 = 0.82 \text{ and } p \leq 0.01 \text{ (t ha}^{-1}\text{)} \quad (1)$$

$$\text{GY} = 0.016\text{NHI}^2 + 2.9\text{NHI} - 122.7 \text{ for } n = 36, R^2 = 0.85, p \leq 0.01 \text{ (t ha}^{-1}\text{)} \quad (2)$$

The second equation indicates that an N–HI of 92.4% would give a yield of 11.22 t ha^{-1}. The maximum yield of 11.18 t ha^{-1} was obtained when the NHI reached 88%. This effect was recorded in 2014 on the Mgs25 plot and Mgf at BBCH 49/50. N–HI was negatively correlated with the amount of N in crop residues and with NUA–G and NUA–T. The highest positive relationships were recorded with PFP–N (r = 0.91 ***) and NAE (r = 0.81 ***).

Magnesium fertilization, as previously documented, significantly affected its accumulation in wheat, but its total accumulation (Mg_aT) was sensitive to the interaction of experimental factors with years (Table A3). The most significant impact of Mg_aT was recorded for N_aCR:

$$N_aCR = -4.16Mg_aT + 111.4 \text{ for } n = 36, R^2 = 0.70 \text{ and } p \leq 0.01 \text{ (kg ha}^{-1}\text{)} \quad (3)$$

The increase in Mg_aT decreased the N accumulated in crop residues. The response of the NUE indices to Mg_aT, as well as to Mg_aG as a major part of Mg_aT, was very specific. A decrease was noted for NUA–G, NUA–T, and N-R (Table A3). An increase was recorded for NUP–G, NUP–T, and N–PhE. PFP–N and NAE were significantly correlated with each other and deserve special attention. PFP–N responded positively but weakly to the amount of Mg_aG or Mg_aT. In contrast, NAE showed a highly positive, linear response to the Mg_aT (Figure A4):

$$\text{NAE} = 1.34Mg_aT + 8.93 \text{ for } n = 36, R^2 = 0.67 \text{ and } p \leq 0.01 \text{ (grain kg kg}^{-1}\text{ Nf)} \quad (4)$$

At the same time, NAE was significantly correlated with both NUP indices but especially with NUP–T (r = 0.84 ***) (Table A2).

3. Discussion

3.1. Magnesium Use Efficiency

An increase in the yields of crop plants in response to the applied nutrient, regardless of the method of application, is the essence of the application of any fertilizer [33]. An assessment of wheat's response to Mg fertilization requires answers to three basic questions:

1. Is there an advantage of soil over foliar Mg fertilization?
2. Does the stage of wheat development affect the choice of the date for foliar Mg fertilization?
3. Is there any interaction between the two fertilization systems with regard to the end result, i.e., yield increase?

The net increase in the yield of winter wheat due to the different systems of Mg fertilization was significant, regardless of the course of the weather over the studied years. In general, the increase in the yield resulting from the single-stage foliar application of Mg was only slightly higher than that of soil-applied Mg (0.52 vs. 0.57 t ha^{-1}). The increase in wheat yield as a result of Mg foliar fertilization increased up to 0.7 t ha^{-1} but provided its double application at BBCH 30 and repeated at BBCH 49/50. The end of booting/beginning of heading is considered the optimal stage for wheat foliar Mg fertilization [26,27]. This stage is crucial for the number of grains per unit area (GD) [34]. In the presented case, the recorded increase in the yield resulted directly from the increase in the GD [32]. The best effect of Mg application on wheat, resulting in a yield gain of 0.92 t ha^{-1}, was due to the interaction of both Mg fertilization systems. The course of weather during the growing season did not markedly change the trend of the wheat response to the tested Mg fertilization systems. On this basis and according to the data in the literature, three strategies for wheat fertilization with Mg during growth can be identified:

1. Conservative: a high and stable yield increase, increasing the resistance to abiotic stresses;
2. Effective: a moderate-to-high yield increase, provided there are no abiotic stresses;
3. Prophylactic: a moderate yield increase, with a constant fertilization factor.

The first strategy was very efficient in years with some disturbances in the weather course during the spring growing season of winter wheat (Figure 1). It requires, however, a higher dose of the in-soil applied Mg and its frequent application to wheat foliage [14,19,20]. The yield-forming effect of the applied Mg can be explained both by the increase in the GD and the maintenance of the photosynthetic activity of leaves during the grain-filling period [24,25,32]. The second Mg fertilization strategy can be recommended in the years or regions of the world with favorable growth conditions for winter wheat. A sufficiently high yield increase can be achieved by applying relatively low in-soil Mg doses, provided a high solubility of fertilizer is used as well as one-stage foliar fertilization with Mg [19,26]. The third fertilization strategy of winter wheat with Mg is based on a double-stage foliar fertilization, regardless of the weather and soil conditions [27].

Mg foliar fertilization was superior to its soil application, but provided a double-stage application, as supported by the obtained data from its extreme efficiency. The values of the Mg recovery indices (Mg–R) on the main Mgf plot ranged from 100% to 300%. In contrast, the Mg recovery on the Mgs plots were in the range of 18–38% for the Mgs25, and 10–19% for the Mgs50 main plots. Moreover, the highest Mg–R indices, regardless of the method of Mg application, were recorded in 2014, the year with the highest grain yield. The extremely high Mg–R values of the Mgf plot were due to the higher Mg uptake by plants and its accumulation in vegetative wheat biomass. The increase in Mg uptake, averaged over treatments, was 16.6% higher compared to the absolute Mg control. It is well documented in the scientific literature that chlorophyll Mg, despite being a stable plant trait, is the main source of N for the growing seed/grain [35]. The obtained results suggest an extension of the grain-filling period (GFP), which resulted in the higher yield. This conclusion is confirmed by Ahnadi-Lahijani and Emam [36], who showed that the higher the chlorophyll content in wheat leaves during GFP and the longer the leaf surface is kept green, the greater the increase in wheat yield.

3.2. Impact of Magnesium Uptake on Nitrogen Management by Wheat

The fourth question concerns the impact of the Mg fertilization system on N management, i.e., N uptake by wheat and its net utilization. The conducted study clearly showed that wheat treated with Mg, regardless of the method of its application, significantly increased the amount of N in wheat at harvest (TN). It should be emphasized that the N taken up by wheat in response to Mg application was mainly accumulated in the grain (Table 2). Both experimental factors contributed to this but without interaction between the systems. The soil-applied Mg increased the amount of N in wheat grain by 24 and 21 kg ha^{-1} in 2014 and 2015. Assuming a protein concentration in the grain at a level of 13%, the corresponding yield increase would be at a level of 1.1 and 0.93 t ha^{-1}. These values are almost equal to the yield increment as a result of the interaction of both mg fertilization systems (Figure 1). Thus, it can be concluded that regardless of weather conditions, the increase in yield is directly related to the efficiency of N utilized by wheat.

The strong relationship between the amount of extra N accumulated in wheat grain, as a result of the of Mg fertilization, was due to the critical role of both nutrients in photosynthesis. Nitrogen is the limiting component of Rubisco, the key plant enzyme responsible for CO_2 fixation [24,25]. The influence of Mg on the activity of Rubisco increases in conditions of water shortage, which is often accompanied by elevated temperatures [36,37]. Rubisco activity increases during the GFP of wheat growth, as recently documented by Shao et al. [25]. This specific effect was probably observed in 2015 on the main plot fertilized with 50 kg Mg ha^{-1} and double Mg fertilization. The stabilizing effect of the soil-applied Mg on the yield of crops is well documented for various biologically different plants such as maize and sugar beets [28–31]. The assumed stabilization results from a plant's accessibility to the readily available Mg, even from the beginning of growth [19,26]. In wheat, the critical period of yield formation starts with the beginning of the elongation phase [8,9]. However, *the critical window* for wheat grain density takes place during the booting and heading stages [8,38]. Foliar Mg fertilization at these stages results in a higher grain density in cereals and maize [27].

Out of the nine studied NUE indices, only four were significantly related to the yield of wheat. The highest relationship was found for the nitrogen harvest index (N–HI). This index is a rule treated as a conservative wheat trait [39]. The study clearly showed its significant dependence on the total amount of Mg in wheat at harvest. The effect of Mg on N–HI was indirect, i.e., it reduced the amount of N in wheat residues. This trend means a greater transfer of N during GFP from the vegetative parts of wheat to the growing grains. The proposed explanation confirms an earlier study by Potarzycki [40,41]. The author showed that foliar Mg applied to wheat at the beginning of the booting phase increased the remobilization of N from vegetative tissues to grain.

Nitrogen apparent efficiency (NAE) was the second-most important ($p \leq 0.01$) NUE index, showing a significant relationship with the yield. It also depended on the total amount of Mg in the wheat biomass or grain (Figure A4). The positive relationship between this index and wheat grain yield indirectly indicates a higher yield from plots fertilized with Mg. Moreover, this index was inversely correlated with the amount of N accumulated in wheat residues but positively with N–HI, NUP–G, NUP–T and, finally, N–PhE. On the basis of the obtained relationships, it can be concluded that, regardless of the fertilization system, Mg had a strong, significant effect on N utilization by winter wheat (Figure A4). The positive effect of Mg on the productivity of N_f was confirmed by the increased productivity of applied fertilizer N, as confirmed by the response of the PFP–N_f index. Its values ranged from 45 kg grain kg^{-1} N_f in 2013, an unfavorable year for wheat growth, to 58 kg grain kg^{-1} N_f in 2014, the year with the highest yields. The highest PFP-N_f values for an N_f rate of 190 kg N ha^{-1} are comparable to those presented by Szczepaniak et al. [42] for wheat fertilized with 160 kg N ha^{-1}. The effective use of N_f by winter wheat through the use of

Mg is emphasized by the values of the nitrogen recovery index (N–R). It was very high on the Mg absolute control plot (NPK only), well above 80%. The use of Mg raised its values above 90%. Moreover, the impact of Mg on this index was very stable, regardless of the weather conditions during the growing season and the method of Mg application.

4. Materials and Methods

4.1. Experimental Site

A field experiment was carried out at Jarosławiec (52°15′ N, 17°32′ E, Poland) on soil originated from sandy loam, classified as Albic Luvisols (Neocambic) [43]. The content of organic matter (C_{org}) in a 0.0–0.3 m layer during the study ranged from 21 ± 0.1 to 25 ± 0.9 g kg^{-1} soil (losses on ignition). Soil reaction (pH) was in the neutral range (1 M KCl). The content of available nutrients, measured before the application of fertilizers was, in general, good for P and sufficient for K and Mg. The amount of the mineral N (N_{min}), determined in a 0.0–0.9 m layer, was high in the first two growing seasons and medium in the third (Table 3).

Table 3. Soil characteristics of the experimental plots during the 2012–2015 growing seasons.

Year	Soil Layer	pH	P [1]	K [1]	Mg [2]	N_{min}
	(cm)	1 M KCl	mg kg^{-1} Soil			kg ha^{-1}
2012/2013	0–30	6.5	63.2 [1] M [3]	157.7 M	33.2 H	76 [4]
	30–60		59.7 [1] M	107.9 M	25.3 M	
2013/2014	0–30	6.7	91.6 [1] H	168.0 M	30.2 M	74
	30–60		91.6 [1] H	153.6 M	24.7 M	
2014/2015	0–30	6.6	87.2 [1] H	182.6 H	24.1 M	57
	30–60		95.9 [1] H	149.9 M	30.2 M	

[1] Egner–Riehm method; [2] Schachtschabel method; [3] classes of the available nutrient content: M—medium, H—high; [4] layer: 0–90 cm (measured in 0.01 M $CaCl_2$).

4.2. Weather Conditions

The weather conditions were very variable in the consecutive growing seasons (Figure 7). The beginning of spring, with the exception of 2012/2013, favored the growth of wheat. In 2013, negative temperatures in the first two decades of March arrested plant growth. In all years of the study, temperatures during flowering and grain filling were within the ranges optimal for yield development. The sum of rainfall during the spring growing season was as follows: 2013—299.4, 2014—285.2, and 2015—265 mm. In 2015, a shortage of rainfall was revealed, which covered three main phases of wheat development, ranging from shooting to early flowering. The sum of rainfall in this period was 37.6 mm, while in 2013, it reached 88.8 mm, and in 2014, 92.6 mm.

4.3. Experimental Design

The field experiment was arranged as a two-factor split-plot design, replicated 4-fold:

1. Soil-applied magnesium (Mgs): 0, 25, and 50 kg Mg ha^{-1} (acronym: Mg control, Mgs25, and Mgs50);
2. Foliar-applied magnesium (Mgf):
    a. Without application, i.e., Mgf control;
    b. Applied at the BBCH 30 stage (I) (I–BBCH 30);
    c. Applied at the BBCH 49/50 stage (II) (II–BBCH 59/50);
    d. Applied at the BBCH 30/31 and BBCH 49/50 stages; double-stage application (I + II).

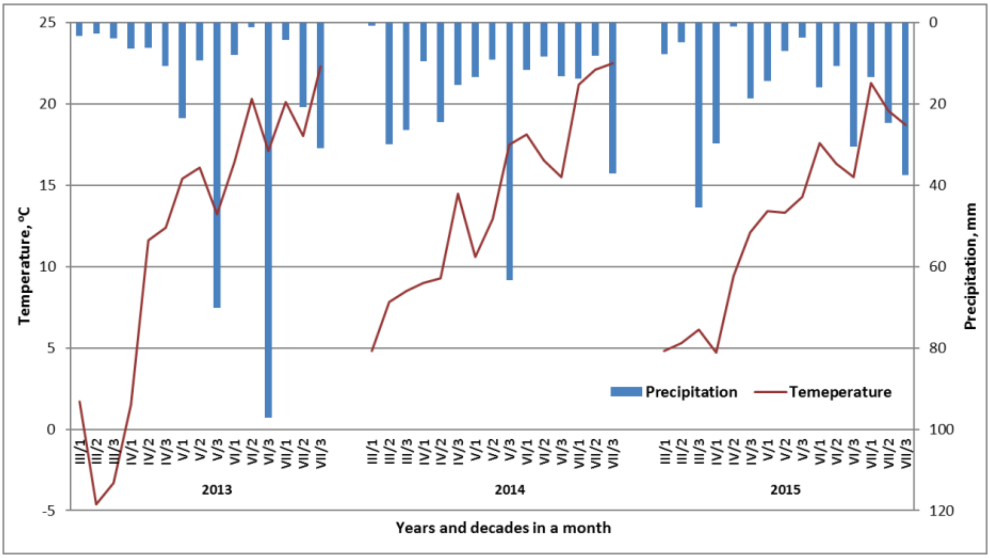

Figure 7. Weather conditions during the consecutive growing seasons.

Spring barley was the fore-crop for winter wheat. The *Tobak* wheat variety was sown annually on 20–25 September. The soil was fertilized with Mg in the form of Kieserite ($MgSO_4 \cdot H_2O$), containing 25% MgO and 50% SO_3. Kieserite was applied to the soil three weeks before wheat sowing. Foliar fertilization of wheat with Mg was carried out using Epsom salt ($MgSO_4 \cdot H_2O$) containing 16% MgO and 37.5% SO_3. The amounts of the applied nutrients are shown in Table 4. Sulfur applied together with the Mg fertilizer was balanced in the first dose of N. It was used as a mixture of ammonium sulfate and ammonium saltpeter (17.5% SO_3). The first N dose of 80 kg ha^{-1} was applied just before the beginning of the growing season in spring. The second dose of 50 kg ha^{-1} was applied, and the third one of 60 kg ha^{-1} at BBCH 45–47. Phosphorus at a rate of 30.1 kg P ha^{-1} as triple superphosphate (46% P_2O_5) and K at a rate of 66.4 kg K ha^{-1} as muriate of potash (KCl) were applied together with the soil Mg. The total area of a single plot was 30 m^2, and the harvested area was 15 m^2. Plant protection was conducted in accordance with the codex of good practice.

Table 4. Fertilization schedule.

Treatment	Fertilization Schedule	$N–P_2O_5–K_2O$	Mg–Soil	Mg–Foliar
			kg ha^{-1}	
1.1	NPK	190–70–80	0	0
2.1	NPK–Mg foliar BBCH 30	190–70–80	0	2.4
2.2	NPK–Mg foliar BBCH 49/50	190–70–80	0	4.0
2.3	NPK–Mg foliar BBCH 30 + 49.50	190–70–80	0	6.4
3.1	NPK–Mg soil	190–70–80	25	0
3.2	NPK–Mg soil + foliar BBCH 30	190–70–80	25	2.4
3.3	NPK–Mg soil + foliar BBCH 49/50	190–70–80	25	4.0
3.4	NPK–Mg soil + foliar BBCH 30 + 49/50	190–70–80	25	6.4
4.1	NPK–Mg soil	190–70–80	50	0
4.2	NPK–Mg soil + foliar BBCH 30	190–70–80	50	2.4
4.3	NPK–Mg soil + foliar BBCH 49/50	190–70–80	50	4.0
4.4	NPK–Mg soil + foliar BBCH 30 + 49/50	190–70–80	50	6.4

4.4. Plant Material Sampling and Analysis

The plant material used for dry matter determination was collected at BBCH 89 from an area of 2.0 m^2. The sampled material was then divided, depending on the wheat stage, into subsamples of grain (G) and crop residues (CRs) composed of leaves (LE), stems (ST), ears (EA), and chaffs (CH). The results are expressed on a dry weight basis.

The N content was determined in both parts of the plant, using the standard macro–Kjeldahl procedure. For determination of the Mg content, the plant sample was dried at 65 °C and then mineralized at 550 °C. The obtained ash was then dissolved in 33% HNO_3. The concentration of Mg was determined using atomic absorption spectrometry—flame type. The results are expressed on a dry matter basis.

4.5. Parameters and Indices of Nitrogen Use Efficiency

The equations used to calculate the amount of N in the grain or crop residues and the N use efficiency (NUE) indices are presented below. The corresponding Mg indices were calculated in the same way.

1. Nitrogen accumulation in wheat grain, *NaG:*

$$N_aG = GY \times N \ \ \text{kg ha}^{-1}$$

2. Nitrogen accumulation in crop residues, N_r:

$$N_aCR_s = CR_s \times N \ \ \text{kg ha}^{-1}$$

3. Total accumulation of nitrogen in wheat biomass, TN:

$$TN = N_aG + N_aCR_s \ \text{kg ha}^{-1}$$

4. Nitrogen harvest index, *NHI:*

$$NHI = \frac{N_aG}{TN} \times 100\%$$

5. Nitrogen unit accumulation in grain, *NUA-G:*

$$NUA = \frac{N_aG}{GY} \ \ \text{kg } N \times t^{-1}$$

6. Nitrogen unit accumulation in total wheat biomass, *NUA-T:*

$$NUA = \frac{TN}{GY} \ \ \text{kg } N \times t^{-1}$$

7. Nitrogen unit productivity—grain, *NUP−G:*

$$NUP - G = \frac{GY \times 1000}{N_aG} \ \ \text{kg } grain \times \text{kg}^{-1} \ N$$

8. Nitrogen unit productivity–total, *NUP-T:*

$$NUP - T = \frac{GY \times 1000}{TN} \ \ \text{kg } grain \times \text{kg}^{-1} \ N$$

9. Partial factor productivity of fertilizer *N*, *PFP-N:*

$$PFP - N = \frac{GY \times 1000}{N_f} \ \ \text{kg } grain \ \text{kg}^{-1} \ N_f$$

10. Nitrogen agronomic efficiency, *NAE*:

$$NAE = \frac{GY_f \times 1000 - GY_{Nc} \times 1000}{N_{if}} \text{ kg } grain \times \text{kg}^{-1} \ N_f$$

11. Nitrogen recovery, *N–R:*

$$N - R = \frac{TN_{N_f} - TN_{N_c}}{N_f} \times 100\%$$

12. Nitrogen physiological efficiency, *N-PhE:*

$$N - PhE = \frac{GY_f \times 1000 - GY_{Nc} \times 1000}{TN_{N_f} - TN_{N_c}} \text{ kg } grain \text{ kg}^{-1} \ N$$

where:

NHI—nitrogen harvest index, %;
CRs—crop residues, t ha^{-1};
N—*N* content in grain or crop residues, %, g kg^{-1} DW;
N_f—plots fertilized with N;
N_c—nitrogen control;
N_f—treatment fertilized with nitrogen.

4.6. Statistical Analysis

The collected data were subjected to an analysis of variance using STATISTICA® 13 (StatSoft, Inc., Krakow, Poland, 2013). The distribution of the data (normality) was checked using the Shapiro–Wilk test. The homogeneity of variance was checked by the Bartlett test. Means were separated by honest significant difference (HSD) using Tukey's method, where the *F*-test indicated significant factorial effects at a level of $p < 0.05$. To determine the wheat grain yield, stepwise regression was used to define the optimal set of wheat components. In the computational procedure, a consecutive variable was removed from the multiple regressions in a step-by-step manner. The best regression model was chosen based on the highest F–value for the model and the significance of all variables.

5. Conclusions

Magnesium applied to wheat resulted in a significant yield gain with respect to the effect of NPK, treated as the Mg control. The method of application was of secondary importance. A slightly higher increase in the yield was caused by foliar fertilization, preferably performed at the booting/heading stages of wheat growth. The yield gain, as a result of foliar fertilization with Mg fertilization, ranged from 0.6 to 0.9 t ha^{-1}, while in the soil, its application resulted in a yield gain in the range of 0.4–0.7 t ha^{-1}. Magnesium accumulation by wheat, averaged for the fertilization treatments, increased by 17% compared to the NPK plot. The recovery of foliar-applied Mg was multiple in relation to the applied dose. The recovery of soil-applied Mg depended on the dose, ranging from 18 to 38% on the 25 kg Mg ha^{-1} main plot and from 10 to 19% on the 50 kg Mg ha^{-1} plot. The main effect of wheat fertilization with Mg was its impact on the uptake and then partitioning of the accumulated N in wheat biomass between the grain and crop residues. The amount of extra accumulated N was effectively converted into grain yield. This process manifested itself in an increase in the value of the nitrogen harvest index and in a decrease in the N content in crop residues.

The main action of Mg, regardless of the weather and the method of its application, was an increase in the productivity of fertilizer nitrogen, which was confirmed by a set of various tested indices such as NUP–G, NUP–T, N–PhE, and PFP–N_f. The yield-forming effect of the applied Mg fertilizer to winter wheat was revealed by the increased N transfer to the grain, which indicates its impact on the nitrogen utilization efficiency.

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Appendix A

Table A1. Matrix of the correlation indices for magnesium accumulation, efficiency indices, and yield, n = 36.

Trait	Mg_aG	Mg_aCR	Mg_aT	Mg–HI	MgUA–G	MgUA–T	MgUP–G	MgUP–T	PFP–Mg	MgAE	Mg–R	Mg–PhE
GY	0.35 *	0.36 *	0.38 *	0.02	−0.33 *	−0.37 *	0.25	0.28	0.07	0.06	0.08	0.07
Mg_aG	1.00	0.67 ***	0.97 ***	0.57 ***	0.77 ***	0.71 ***	−0.82 ***	−0.77 ***	0.01	0.05	0.10	0.07
Mg_aCR		1.00	0.83 ***	−0.23	0.44 **	0.56 ***	−0.48 **	−0.62 ***	−0.06	−0.02	0.02	0.04
Mg_aT			1.00	0.36 *	0.72 ***	0.71 ***	−0.77 ***	−0.78 ***	−0.01	0.03	0.08	0.07
Mg–HI				1.00	0.54 **	0.34 *	−0.58 ***	−0.35 *	0.07	0.09	0.10	0.05
MgUA–G					1.00	0.97 ***	−0.99 ***	−0.97 ***	−0.04	0.01	0.04	0.02
MgUA–T						1.00	−0.96 ***	−0.99 ***	−0.06	−0.01	0.02	0.01
MgUP–G							1.00	0.97 ***	0.01	−0.04	−0.07	−0.05
MgUP–T								1.00	0.04	−0.01	−0.05	−0.04
PFP–Mg									1.00	1.00 ***	0.99 ***	0.95 ***
MgAE										1.00	1.00 ***	0.96 ***
Mg–R											1.00	0.96 ***

***, **, and * indicate significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; n.s.—not significant. Mg_aG, Mg_aCR, and Mg_aT—magnesium accumulation: grain, crop residues, and total, respectively; Mg–HI—magnesium harvest index; MgUA-G and MgUA-T—magnesium accumulation: grain and total, respectively; MgUP–G and MgUP–T—magnesium unit productivity: grain and total, respectively; PFP–Mg—partial factor productivity of fertilizer magnesium; MgAE—magnesium apparent efficiency; Mg–R—magnesium recovery; Mg–PhE—magnesium physiological efficiency.

Table A2. Matrix of the correlation indices for nitrogen accumulation, efficiency indices, and yield, n = 36.

Trait	N_aGY	N_aCR	TN	N–HI	NUA–G	NUA–T	NUP–G	NUP–T	PFP–N	NAE	N–R	N–PhE
GY	0.50 **	−0.53 **	0.26	0.9 ***	−0.16	−0.38 ***	0.22	0.47 **	1.00	0.75 ***	0.14	0.38 *
N_aG	1.00	0.40 *	0.96 ***	0.24	0.78 ***	0.61 ***	−0.73 ***	−0.53 **	0.50 **	−0.10	0.91 ***	−0.61 ***
N_aCR		1.00	0.63 ***	−0.78 ***	0.84 ***	0.94 ***	−0.84 ***	−0.94 ***	−0.53 ***	−0.83 ***	0.70 ***	−0.91 ***
TN			1.00	−0.01	0.90 ***	0.79 ***	−0.83 ***	−0.72 ***	0.26	−0.33	0.97 ***	−0.78 ***
N–HI				1.00	−0.37 *	−0.59 ***	0.40 *	0.64 ***	0.91 ***	0.81 ***	−0.13	0.55 ***
NUA–G					1.00	0.97 ***	−0.99 ***	−0.94 ***	−0.16	−0.67 ***	0.93 ***	−0.97 ***
NUA–T						1.00	−0.97 ***	−0.99 ***	−0.38 *	−0.79 ***	0.85 ***	−0.99 ***
NUP–G							1.00	0.96 ***	0.23	0.68 ***	−0.91 ***	0.98 ***
NUP–T								1.00	0.47 **	0.83 ***	−0.80 ***	0.99 ***
PFP–N									1.00	0.75 ***	0.14	0.38 *
NAE										1.00	−0.37 *	0.80 ***
R–N											1.00	−0.83 ***

***, **, and * indicate significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; n.s.—not significant. [1] I—BBCH 30; II—BBCH 49/50. N_aG, N_aCR, and N_aT—nitrogen accumulation: grain, crop residues, and total, respectively; N–HI—nitrogen harvest index; NUA-G and NUA-T—nitrogen accumulation: grain and total, respectively; NUP–G and NUP–T—nitrogen unit productivity: grain and total, respectively; PFP–N—partial factor productivity of fertilizer nitrogen; NAE—nitrogen apparent efficiency; N–R—nitrogen recovery; N–PhE—nitrogen physiological efficiency.

Table A3. Correlation matrix of magnesium accumulation and indices of nitrogen use efficiency.

Trait	MgaCR	MgaT	NUA-G	NUA–T	NUP–G	NUP–T	PFP–N	NAE	R–N	PhE–N
MgaG	0.67 ***	0.97 ***	−0.91 ***	−0.91 ***	0.90 ***	0.92 ***	0.35 *	0.82 ***	−0.75 ***	0.93 ***
MgaCR	1.00	0.83 ***	−0.54 **	−0.57 ***	0.54 **	0.57 ***	0.34 *	0.68 ***	−0.30	0.58 ***
MgaT		1.00	−0.86 ***	−0.89 ***	0.86 ***	0.88 ***	0.38 *	0.84 ***	−0.67 ***	0.89 ***

***, **, and * indicate significant differences at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively; n.s.—not significant. Mg_aG, Mg_aCR, and Mg_aT—magnesium accumulation: grain, crop residues, and total, respectively; NUA–G and NUA–T—nitrogen accumulation: grain and total, respectively; NUP–G and NUP–T—nitrogen unit productivity: grain and total, respectively; PFP–N—partial factor productivity of fertilizer nitrogen; NAE—nitrogen apparent efficiency; N–R—nitrogen recovery; N–PhE—nitrogen physiological efficiency.

Appendix B

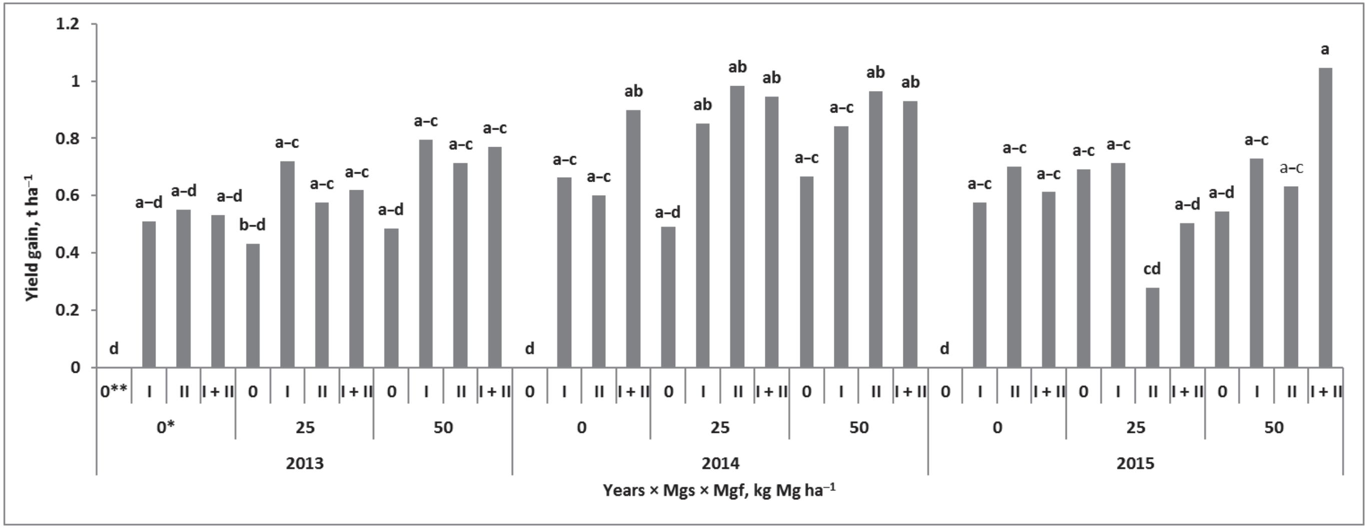

Figure A1. The increase in the yield of winter wheat in response to the interaction of years and Mg fertilization systems. a, b, c, d Similar letters mean a lack of significant differences using Tukey's test. Mgs and Mgf—soil and foliar Mg fertilization systems, respectively. * Doses of soil-applied Mg, kg ha^{-M}; ** stages of Mg foliar fertilization to wheat: I—BBCH 30; II—BBCH 49/50.

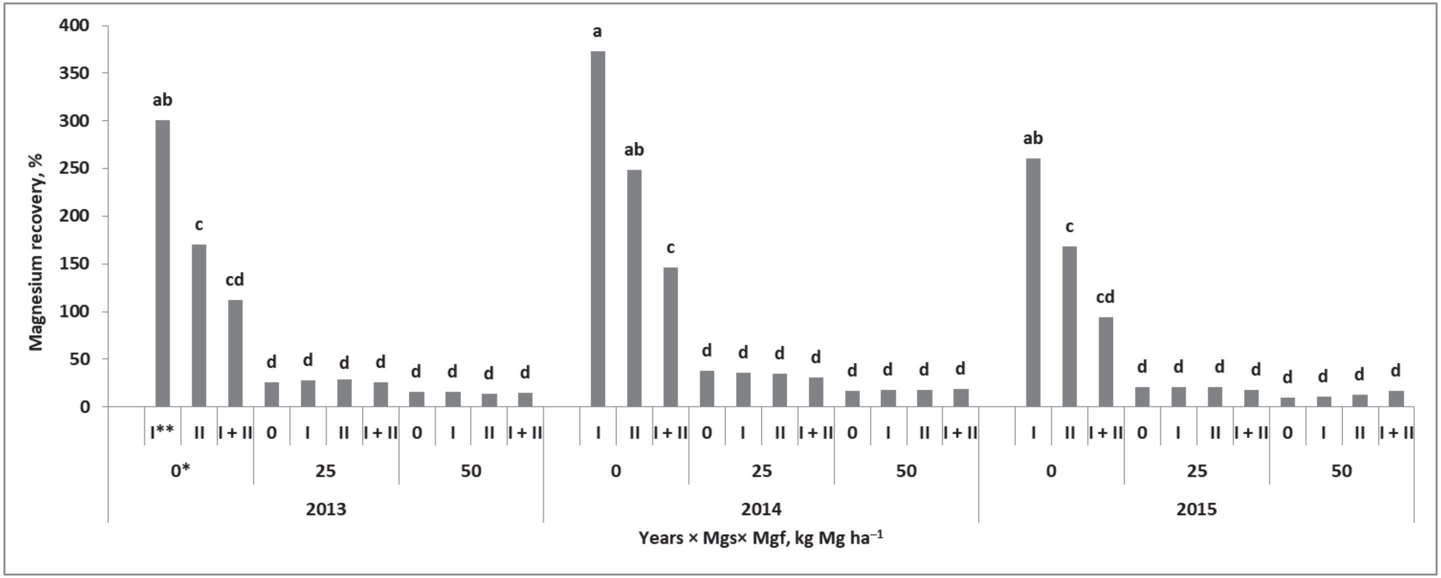

Figure A2. Magnesium recovery index in response to f Mg fertilization systems and years. a, b, c, d Similar letters mean a lack of significant differences using Tukey's test. Legend: Mgs, Mgf—soil and foliar Mg fertilization system, respectively; * doses of applied Mg, kg ha^{-1}; ** stages of Mg foliar fertilization to wheat: IBBCH—30; II—BBCH 49/50.

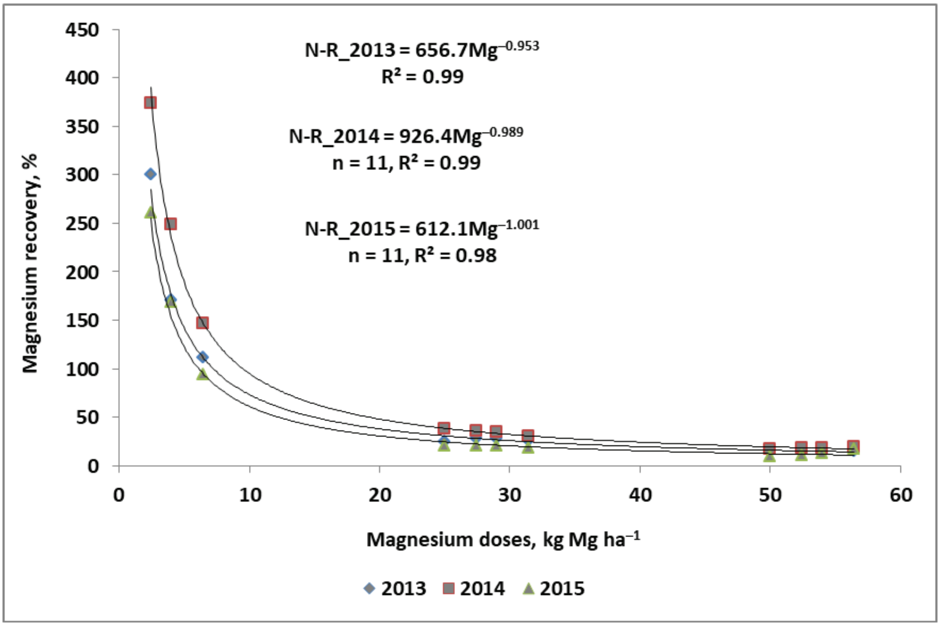

Figure A3. General course of magnesium recovery in consecutive years of study.

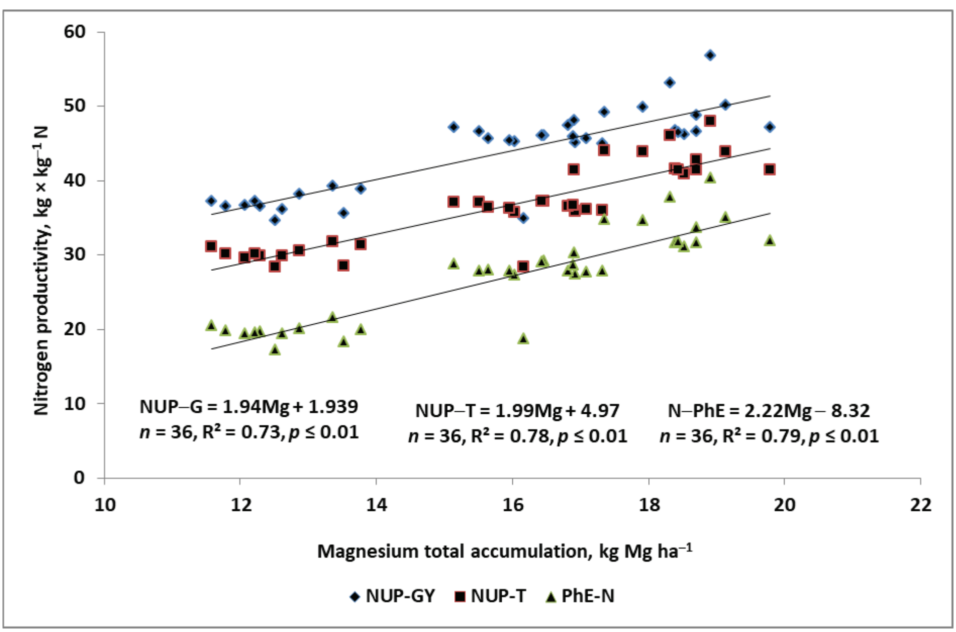

Figure A4. Effect of magnesium total accumulation in winter wheat on indices of nitrogen utilization.

Legend: NUP-G and NUP-T—nitrogen unit productivity: grain and total, respectively; N-PhE—nitrogen physiological efficiency.

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Impact of Foliar Fertilization on Growth, Flowering, and Corms Production of Five *Gladiolus* Varieties

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Abstract: Degraded and salt affected soils are appearing more often in cultivated areas. These specific problems could reduce nutrient uptake, which can result in quality and yield loss of the cultivated plants. In order to cope with this pedo-climatic condition growers are applying fertilizers; however, due to inadequate application, soil degradation will continue. Five *Gladiolus* varieties were subjected to foliar fertilization treatments to assess the effect on the plant's growth parameters, vase durability and daughter corm production. Our results indicate that plants treated with foliar fertilization show significant increase in the measured parameters, flower stem length, vase durability and daughter corm production. In conclusion, our study suggests that application of foliar fertilization can increase *Gladiolus* plants decoration and propagation, even with a smaller footprint on nature.

Keywords: corms; foliar fertilization; *Gladiolus*; vase durability

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1. Introduction

Currently in the world 20% of the cultivated land is degraded and salt affected, which is affecting nutrient uptake and resulting in quality and yield reduction of the cultivated plants. More importantly, these factors are contributing to crop losses worldwide [1,2]. According to several studies, in order to cope with these conditions and increase production, chemical fertilizers are applied, but due to inappropriate application, soil degradation (acidification; salinization; nutrient imbalance; and irregular accumulation of nitrogen, phosphorus and potassium) occurs in the cultivated lands [3,4].

Fertilization can be divided into two main methods: root and foliar fertilization [4]. Foliar fertilization can be absorbed directly through the leaves and can be transported more quickly and efficiently to the other plant organs compared to root fertilization [5,6]. Moreover, foliar fertilization can be sprayed at optimum times and concentrations, according to the requirement of different plants, at different growth stages. This type of fertilization can be more suitable to the plant's needs, in contrast to root fertilization [4,7,8].

Gladiolus genus is a perennial, monocotyledonous, geophyte, semi-rustic ornamental plant and includes about 260 species [8,9]. The *Gladiolus* originates in Mediterranean Europe, Asia, and South and Tropical Africa [10–13]. It can be found as an ornamental garden plant and also as cut flowers used for bouquets and arrangements [13]. These majestic plants are found in different shapes, colours, and sizes, and can be cultivated almost everywhere, but should be considered for regions where spring and summer conditions are favourable [14].

The main aim of the present research was to test the responses to foliar fertilization of five highly cultivated *Gladiolus* varieties. 'Black Beauty', 'Green Star', 'Nova Lux', 'Zizane', and 'Frizzled Coral Lace' were analysed in the study. The effect of three foliar fertilizers—Fitofolis, Bionat Plus and Cropmax—and the mixture of the three on the flower quality and the amount of new daughter corms produced by the selected gladioli was investigated. We expected to establish whether any of the five varieties was more suitable for cultivation in the climate conditions of the Carpathian Basin.

2. Results

2.1. Plant Growth

Considering the shoot growth, almost all *Gladiolus* varieties showed small increases in shoot length (Figure 1). However, in the case of 'Green Star' (Figure 1a) the Fitofolis and the F + B + C (the mixture of Fitofolis, Bionat Plus and Cropmax) treatments influenced the shoot growth significantly compared to the control treatments. The effect of the treatment was evident, especially for the 'Black Beauty' gladioli, which showed an increase in growth in all treatments with respect to the controls, with ~10 cm (Figure 1b).

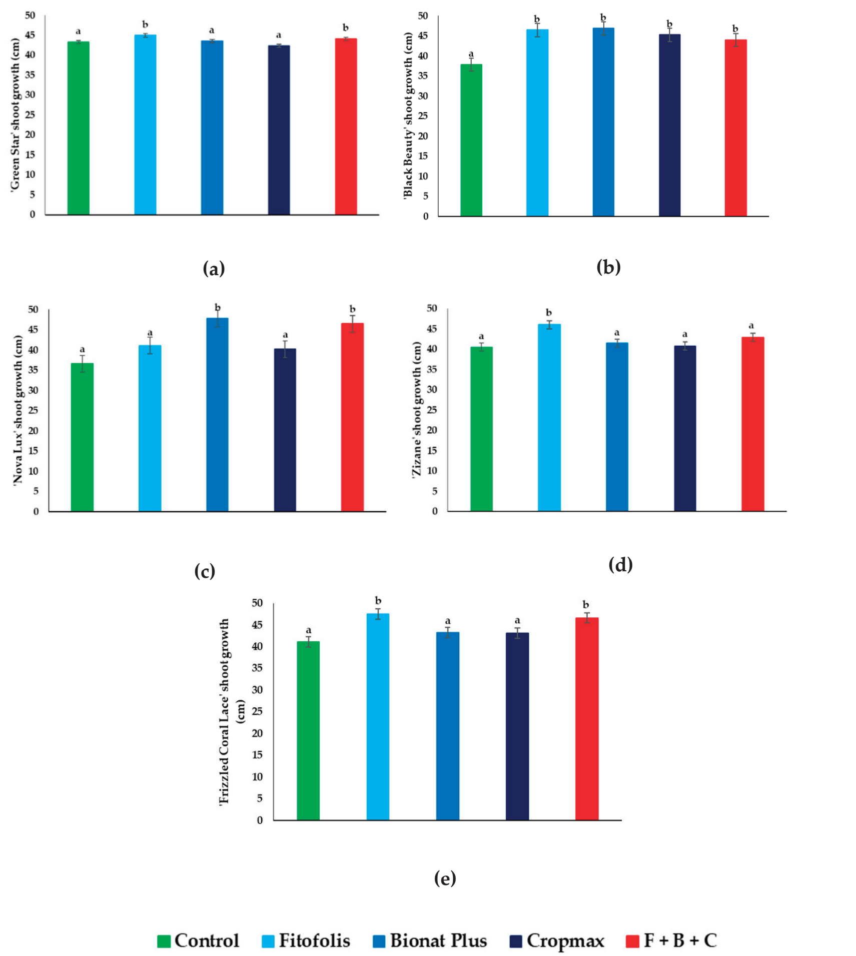

Figure 1. Effect of foliar fertilization on the shoot growth parameters in *Gladiolus* varieties: 'Green Star' (**a**), 'Black Beauty' (**b**), 'Nova Lux' (**c**), 'Zizane' (**d**), and 'Frizzled Coral Lace' (**e**). Plants shoot growth under control conditions, in the presence of the indicated foliar fertilization: Fitofolis, Bionat Plus, Cropmax and the mixture of Fitofolis–Bionat Plus–Cropmax (F + B + C). Shoot growth was measured in all plants just before starting the treatments (13 May), and before the harvesting of the inflorescences (9 August). Bars represent the means ± SE (n = 10). Different letters above the bars indicate significant differences between the treatments, according to Tukey test (α = 0.05).

Regarding 'Nova Lux' (Figure 1c), only for the Bionat Plus and F + B + C treatments were significant differences reported; however, all treatments showed small increases (between 5–10 cm) compared to the control plants. An increase in mean shoot growth was also reported in 'Zizane' for the Fitofolis treatment (Figure 1d); for 'Frizzled Coral Lace' (Figure 1e), an increase of approximately 4 cm, was observed for the Fitofolis and F + B + C treatments.

Percentage increases in shoot growth of *Gladiolus* varieties influenced by the foliar fertilization were as follows: for 'Green Star', 'Zizane', and 'Frizzled Coral Lace' the highest percentage increase was recorded with Fitofolis (3.92%, 13.58%, and 15.54%) compared to the control. In contrast, the smallest percentage increases for the same three varieties were observed with Cropmax (0.02%, 0.61%, and 4.86%). The 'Black Beauty' reported the highest increase with Bionat Plus (23.81%) and the smallest percentage increase at F + B + C (16.27%). For 'Nova Lux', a 30.6% increase was found with Bionat Plus compared to only a 9.8% increase with Cropmax fertilization.

The results of the present study indicated that a variety-specific response exists to foliar fertilization; in most cases the treatments significantly, positively influenced the flower stem growth (Figure 2) compared to the controls.

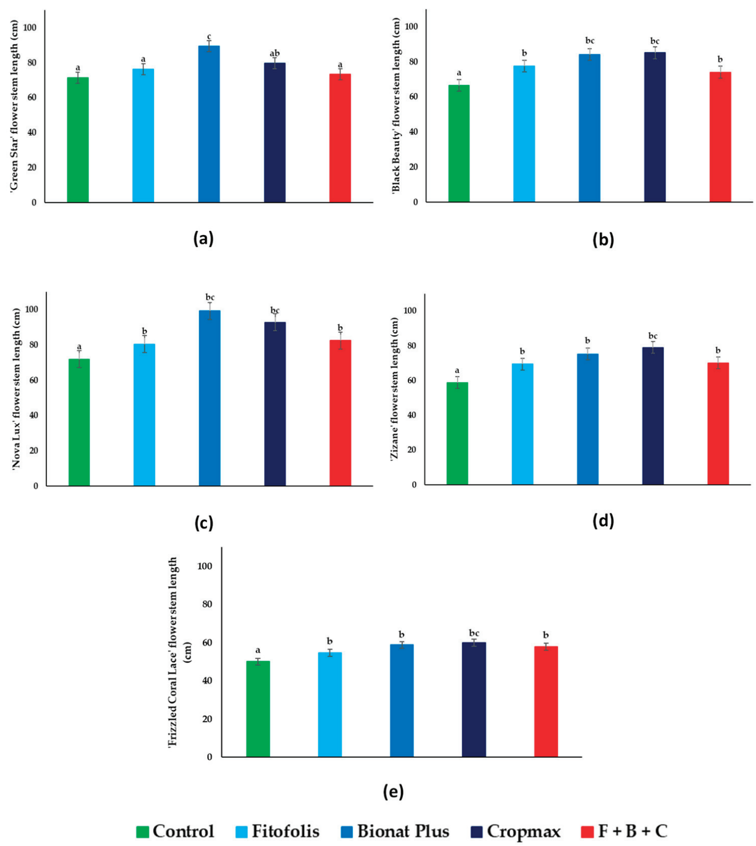

Figure 2. Effect of foliar fertilization on flower stem growth parameters in *Gladiolus* varieties: 'Green Star' (**a**), 'Black Beauty' (**b**), 'Nova Lux' (**c**), 'Zizane' (**d**), and 'Frizzled Coral Lace' (**e**). Plant flower stem growth shown under control conditions and in the presence of the indicated foliar fertilization: Fitofolis, Bionat Plus, Cropmax and the mixture of Fitofolis–Bionat Plus–Cropmax (F + B + C). Flower stem growth was measured in all plants just before starting the treatments (13 May), and before the harvesting of the inflorescences (9 August). Bars represent the means ± SE (n = 10). Different letters above the bars indicate significant differences between the treatments, according to Tukey test (α = 0.05).

'Green Star' (Figure 2a) showed significant growth of the flower stem under the Bionat Plus fertilization, with a 19 cm increase; the other fertilization treatments influenced the flower stem growth, but at smaller percentages.

All types of foliar fertilization increased flower stem length for 'Black Beauty' (Figure 2b), compared to the control plants, in some cases by almost 20 cm. Regarding 'Nova Lux', 'Zizane' and 'Frizzled Coral Lace' *Gladiolus* (Figure 2c–e), similar results were found for all four fertilization treatments. For these three *Gladiolus* varieties, growth increase was between 5 and 30 cm depending on the treatment.

Comparing the stem growth between varieties in all treatments, the highest increase was observed in 'Nova Lux'—almost 30 cm; the least growth was in 'Frizzled Coral Lace'. This could be explained by the variety morphology.

When comparing the flower stem growth to the control, the greatest percentage increases in 'Black Beauty', 'Frizzled Coral Lace', and 'Zizane' were recorded with Cropmax (27.96%, 19.76%, and 34.35%), in 'Nova Lux' and 'Green Star' with Bionat Plus with 37.91% and 25.07%, respectively. The lowest percentage increases were observed with Fitofolis ('Nova Lux'–11.82%, 'Frizzled Coral Lace'–9.18%, and 'Zizane'–18.02%) and F + B + C ('Black Beauty'–11.27% and 'Green Star'–2.66%) fertilizers.

2.2. Vase Durability

Under our experimental conditions, significant differences between the varieties and the treatments were observed in the vase durability of the *Gladiolus* (Figure 3). When comparing the varieties, it could be concluded that 'Green Star', 'Black Beauty' and 'Nova Lux' had the longest vase durability, whereas 'Zizane' and 'Frizzled Coral Lace' had shorter vase durability, with fewer points.

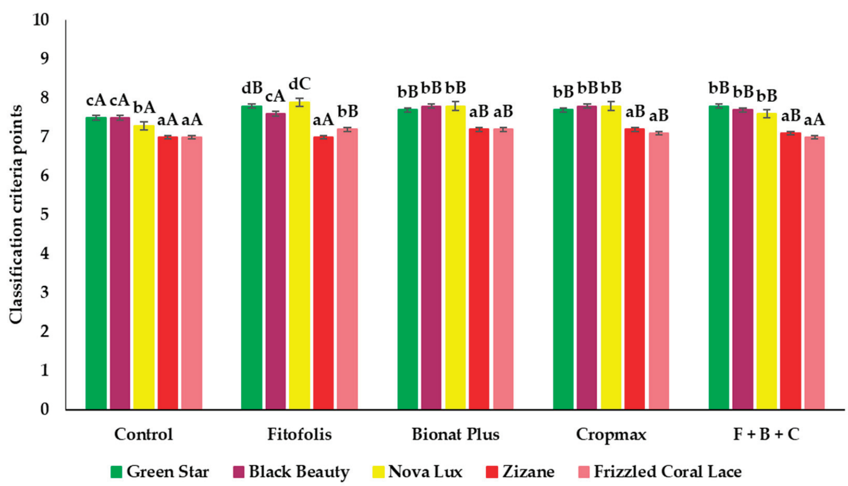

Figure 3. Effect of foliar fertilization on vase durability of *Gladiolus* varieties: 'Green Star', 'Black Beauty', 'Nova Lux', 'Zizane', and 'Frizzled Coral Lace'. Vase durability of floral stems produced under control conditions and in the presence of the indicated foliar fertilization: Fitofolis, Bionat Plus, Cropmax and the mixture of Fitofolis–Bionat Plus–Cropmax (F + B + C). Bars represent the means ± SE (n = 5). Different lowercase letters above the bars indicate significant differences between the five varieties for each foliar fertilization, and different uppercase letters indicate significant differences between treatments, according to Tukey test (α = 0.05).

Foliar fertilizations influenced vase durability in a positive way, although with small differences. Almost all types of fertilization affected the gladioli durability, supporting the general conclusion of the individual experiments that all varieties responded to fertilization. The average vase durability was 7.45 days.

2.3. Daughter Corms Production

It was concluded that foliar fertilization had a positive effect on the increase in number of daughter corms production.

Under our experimental conditions 'Green Star' and 'Black Beauty' showed significant increases from all types of foliar fertilization, compared to the controls (Figure 4a,b).

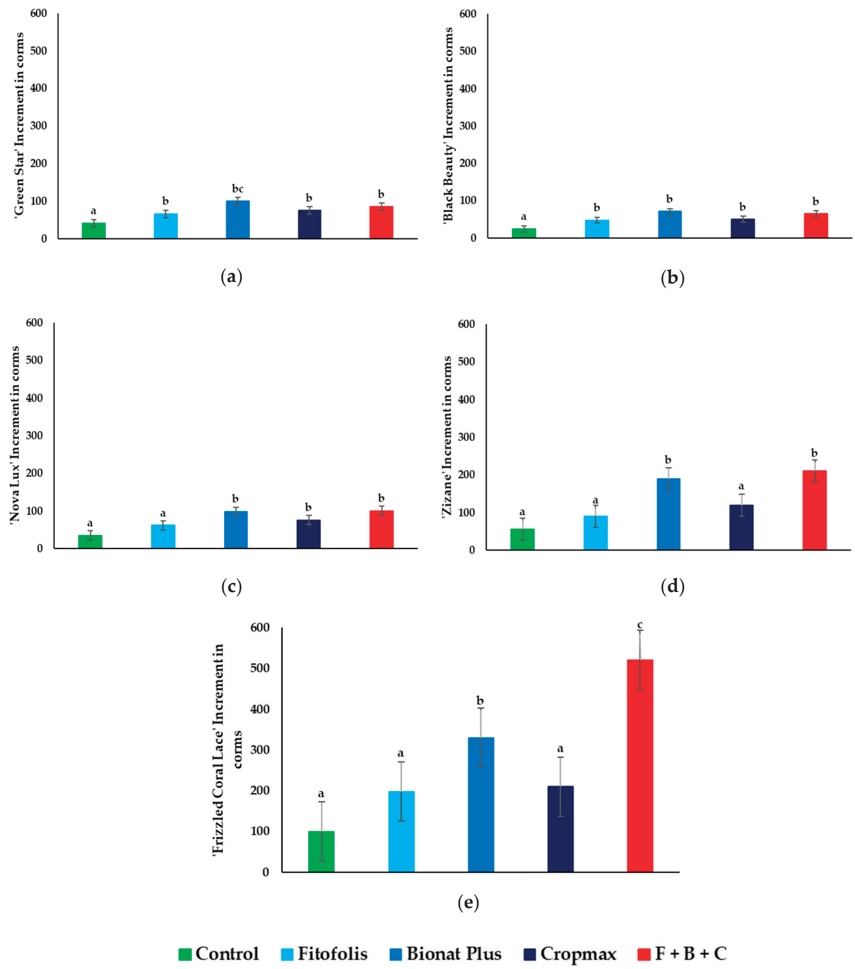

Figure 4. Effect of foliar fertilization on increment in daughter corms in *Gladiolus* varieties: 'Green Star' (**a**), 'Black Beauty' (**b**), 'Nova Lux' (**c**), 'Zizane' (**d**), and 'Frizzled Coral Lace' (**e**). Increase in daughter corms under control conditions and in the presence of the indicated foliar fertilization: Fitofolis, Bionat Plus, Cropmax and the mixture of Fitofolis–Bionat Plus–Cropmax (F + B + C). Bars represent the means ± SE (n = 10). Different letters above the bars indicate significant differences between the treatments, according to Tukey test (α = 0.05).

For 'Nova Lux' (Figure 4c), there was no effect from the Fitofolis treatment. In contrast, the Bionat Plus, Cropmax and F + B + C treatments increased the corms production. Increases in the number of corms were also observed in 'Zizane' (Figure 4d) with the Bionat Plus and F + B + C treatments.

'Frizzled Coral Lace' (Figure 4e) showed a high increase from the F + B + C treatment, as daughter corms production was five times higher compared to the controls. This result could be influenced also by the variety: comparing the five different *Gladiolus* varieties, the greatest daughter corm production occurred in this variety.

In the cases of 'Green Star' (60.97%), 'Black Beauty' (92%), 'Nova Lux' (77.14%), and 'Zizane' (63.63%) the smallest percentage increase in relation to the controls were reported from Fitofolis. For 'Frizzled Coral Lace', the Cropmax fertilizer recorded the smallest increase in percentage with a 228.12% compared to control. The greatest increases were observed with Bionat Plus ('Green Star'–143.9% and 'Black Beauty'–184%) and the mixture of the three foliar fertilizers ('Nova Lux'–185.71%, 'Zizane'–281.81%, and 'Frizzled Coral Lace'–714.06%).

3. Discussion

The results of this experiment show proper foliar fertilization can support and influence the growth, vase durability and daughter corms production of some *Gladiolus* varieties. Saima et al. [15] found that application of foliar spray affected flower production and it was the best method to getting maximum flower production in *Gladiolus*. Furthermore, it has a potential effect on the nutrient uptake and on the stimulation of growth parameters and flowering characteristics [5,16]. Foliar fertilization increases micronutrient uptake and physiological and biochemical indexes [17,18]. Many studies suggest that foliar fertilization may help to stimulate the uptake of soil applied fertilizers, which could provide a solution to salt accumulation in the soil [4].

Foliar fertilization was more effective and significantly enhanced the shoot and flower stem growth compared to the control plants. Similar to our study, some researchers reported that foliar fertilization promoted the flower stem growth to the maximum levels in gladioli, which could have a constructive role in the development of the flowers [15,19,20]. Similar findings have been described where the administration of foliar fertilization treatments influenced the *Calendula* inflorescence yield, but not the chlorophyll parameters, where no significant differences were observed between the treatments [21,22].

The data obtained clearly show that foliar fertilization can affect shoot growth in a positive way. Furthermore, the Fitofolis fertilizer obtained the best results compared to the control, which in some cases increased the growth up to 5 cm. The mixture of the three fertilizers (F + B + C) influenced shoot growth of gladioli in a positive way. In some varieties increases of 3 cm were shown compared to treatments with only Cropmax or Bionat Plus.

Like the shoot growth parameters, flower stem growth was influenced in a positive way by the foliar fertilization in all five varieties. Generally, the highest increases were observed in the plants fertilized with Bionat Plus, followed by Cropmax and Fitofolis. The mixture of foliar fertilizers in this case did not record as high an increase compared to the other three treatments. The macronutrients (N, P and K) are known to have effect on plant growth [23]. Nitrogen, phosphorus and potassium influenced the shoot growth and the flower stem length in a positive way. NPK used at an optimal dose can supplement sufficient nutrient uptake, which foster conditions for plants growth and development [24]. In some studies, it was also reported that B (boron) could increase–stimulate nutrient uptake, maintaining cell integrity and intensify respiration rate, which could promote growth and flower development [25–27].

Vase durability of *Gladiolus* is one of the most important considerations for consumers. Foliar fertilizer effects on vase durability have been reported on *Rosa* [28], *Lilium* [29], *Anthurium andreanum* [30] and *Gladiolus* [31,32]. In the present experiment, vase durability of the five gladioli varieties was improved compared to the control in almost all treatments. The longest vase durability was obtained under the Bionat Plus treatment, and the longest vase durability among the five varieties was observed for 'Green Star'. *Gladiolus* fading or wilting are important signalling factors of senescence [33]. Calcium (Ca) has an important role in regulating the senescence in gladioli cut flowers [34]. Ca increases membrane stability and reduces the level of reactive oxygen species, which could delay senescence in *Gladiolus* cut flowers [35]. However, in a study conducted by Dhakal et al. [31] it was concluded that phosphorus could also improve vase durability of gladioli cut flowers.

Daughter corm production is an important part of the gladioli propagation; our study results clearly indicate foliar fertilization has an important role in this sequence of the cultivation. Previous reports have also shown an increase in daughter corms production under foliar fertilization treatments [16,36–38]. Under our experimental conditions the highest increase was recorded in 'Frizzled Coral Lace' compared to the other four *Gladiolus varieties*. The mixture foliar fertilization (F + B + C) improved the daughter corm production. This behaviour could be explained also with the variety characteristics, where previous results reported 'Frizzled Coral Lace' has a high yield of daughter corms. Daughter corm production could be influenced by nitrogen dose [39,40], and some studies have shown that daughter corm production is also influenced by applying a higher potassium dose [15,16,41].

4. Materials and Methods

4.1. Experimental Site and Plant Material

Open field experiments were conducted between April and September 2018 at the Sapientia Hungarian University of Transylvania, Târgu Mureș (46°31′17″ N 24°35′54″ E). The gladioli corms were obtained from Sieberz Garden Centre (Gödöllő, Hungary) and planted in five rows/block, each row containing 10 gladioli corms, with sizes of 12–14 cm in circumference. According to the soil analysis and of the analysis of its profile we can state that the type of soil at the experiment location is gley chernozem, carbonated in depth and clayish in the alluvial deposits (Epiaquic Hapludalfs) (Table 1).

Table 1. Planted soil proprieties.

Depth (cm)	pH	P (ppm)	K (ppm)	Humus %	N %	Cohesion Coefficient	$CaCO_3$ (ppm)
0–10	7.55	586	1850	7.29	0.348	48	1.33
10–20	7.48	520	1630	7.12	0.33	48.4	1.25
20–40	7.44	487	1550	6.22	0.304	48.8	

The average temperature during the experiment was 17.99 °C, the minimum was recorded in April (15.4 °C), and the maximum temperature in August (21.83 °C) (Figure 5). From Figure 5, it can be concluded that the average precipitation amount was 54.83 mm during the experimental months. The minimum precipitation was recorded in April at 15.40 mm, and the maximum in June was 129.40 mm. The precipitation and temperature data were collected using Delta–T devices WS–GP2 Automatic Weather Station (Delta-T Devices Ltd., Burwell, UK).

Morphological description of the five selected *Gladiolus* varieties:

- 'Black Beauty': flower colour dark burgundy; usually grows a flower stalk that is thin, straight, stiff; plant height 70–90 cm.
- 'Green Star': flower colour lime green; usually grows a flower stalk that is straight and vigorous; plant height 75–100 cm.
- 'Nova Lux': flower colour bright yellow; the flower stalk is thin but strong; plant height 80–110 cm.
- 'Zizane': flower colour combination of red and white; grows a straight, strong flower stalk; plant height 80–110 cm.
- 'Frizzled Coral Lace': flower colour coral pink; tends to grow more flower stems; plant height 60–100 cm.

Gladiolus corms were planted on 18 April 2018 with a row length of 25 cm and 15 cm between the plants. The plant growth was already observed at the end of the planting month.

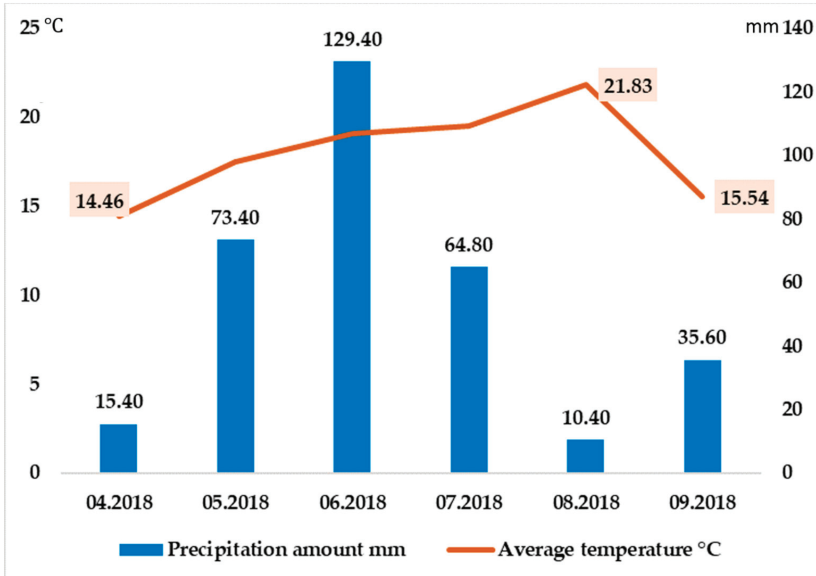

Figure 5. Meteorological conditions, precipitation and temperature during the field experiment (April–September 2018).

4.2. Application of the Foliar Fertilization

On 13 May, two weeks after sprouting, first shoot measurements and the first foliar fertilization were made, according to the experimental design: A–Control, B–Fitofolis (Chemtech, Târgu Mureș, Romania), C–Bionat Plus (Panetone, Timișoara, Romania), D–Cropmax (Blondy, Târgu Mureș, Romania) and E–the mixture of the three foliar fertilizers (first it was fertilized with Fitofolis, the second fertilization was made with Bionat Plus, the third with Cropmax, and the last one with the mixture of the three fertilizers in 1:1:1 proportion) (Table 2).

Table 2. Planting design: A: Control; B: Fitofolis; C: Bionat Plus; D: Cropmax; E: The mixture of Fitofolis, Bionat Plus and Cropmax (F + B + C).

A	B	C	D	E
Green Star	Black Beauty	Zizane	Frizzled Coral Lace	Nova Lux
Nova Lux	Green Star	Black Beauty	Zizane	Frizzled Coral Lace
Frizzled Coral Lace	Nova Lux	Green Star	Black Beauty	Zizane
Zizane	Frizzled Coral Lace	Nova Lux	Green Star	Black Beauty
Black Beauty	Zizane	Frizzled Coral Lace	Nova Lux	Green Star

The used foliar fertilizers content:

- Fitofolis: N–183 g/L; P–43 g/L; K–46 g/L; Fe–0.4 g/L; Cu–0.06 g/L; Mn–0.086 g/L; B–0.01 g/L; Zn–0.05 g/L; Mo–0.004 g/L.
- Bionat Plus: N–6.9%; P–0.003%; K–0.76%; Mg–0.47%; S–1.3%; Ca–0.05%; B–0.16%; Cu–0.35%; Fe–0.18%; Mn–0.06%; Zn–0.11%.
- Cropmax: N–0.2%; P–0.4%; N–0.02%; Fe–220 mg/L; Mg–550 mg/L; Zn–49 mg/L; Cu–35 mg/L; Mn–54 mg/L; B, Ca, Mo, Co, Ni–10 mg/L; auxin; cytokinin; gibberellin.

The application of the foliar fertilizers was done with a hand sprayer; for each product a 2% solution was prepared. To prevent the fertilization from getting into the wrong row, we placed a plastic film between the rows to protect them from the other treatments.

On 2 June the second foliar fertilization was applied; the only difference was that the gladioli on bed E were sprayed with the Bionat Plus. The third fertilization was made on 20 June, for the last (E) gladioli bed, Cropmax foliar fertilizer was applied.

The last fertilization was done on 5 July; E bed was fertilized with the mixture of the three foliar fertilizations (Fitofolis, Bionat Plus, Cropmax) in 1:1:1 proportions. The first flower buds appeared on 13 July. We measured the flower stem length of each plant. The last shoots measurements were made before harvesting the five *Gladiolus* varieties (9 August).

4.3. Vase Durability

When almost all gladioli started flowering (11 August) we randomly harvested five flowered stems, at a cut distance of 5 cm above the soil, from each treatment/*Gladiolus* variety. The vase durability was studied for seven days, and the gladioli cut flowers were kept under the same conditions: at room temperature, in clean-fresh water, and monitored and noted every three hours.

The classification criteria for the vase durability were determined as follows:

- Ten points if the flower buds are healthy, the lower inflorescence is fully open, the colour is bright and typical for the variety, the stem is flexible and straight, the leaves are brightly coloured.
- Nine points if the second and third flower buds have opened, the petal edges on the first are starting to wilt slightly, the stem is losing a little of its hold.
- Eight points when the fourth and fifth flower buds have opened, the petals of the lowest flower start to wilt.
- Seven points if lowest flower fully open, withered, leaves completely lose their glossy colour, stem loses elasticity.
- Two points when the uppermost buds in the inflorescence is opened, and the ones underneath are constantly wilting.

The above was used to determine the maximum value of vase durability.

4.4. Corms Propagation

At the end of August, all inflorescences were harvested from the plants; however, we left two leaves on each *Gladiolus* plant, thus contributing to the growth of the corms. In this way most of the photoassimilation is destined for the growth of the daughter corms. By the end of September, when the plants had stopped nutrient uptake and the remaining leaves withered, we gathered corms of five *Gladiolus* varieties. We dried them and cleaned the remaining soil of the corms.

4.5. Statistical Analysis

All data were tested for normality of errors and homogeneity of variance. As all data were normally distributed, ANOVA followed by Tukey test were used to compare variances. The significance of the differences between the treatments was tested by applying two-way ANOVA, at a confidence level if 95%. When the ANOVA null hypothesis was rejected, Tukey's Post hoc test was carried out to establish the statistically significant differences at $p < 0.05$.

5. Conclusions

Gladioli growers strive to achieve the greatest possible stem length, vase durability and daughter corm production. The present study provides new experimental data on the responses of five *Gladiolus* varieties to foliar fertilization. For flower stem length and vase durability increase we recommend the use of Bionat Plus fertilizer, and for cut flowers the 'Green star' variety, which in our experiments had the best increases. The highest yield of daughter corm production was observed with the mixture of the three foliar fertilizations (F + B + C). These approaches/results will help to enhance the production of *Gladiolus*, with a smaller footprint on the degradation and salinization of the cultivated lands. Due to the frequent changes in cultivated *Gladiolus* varieties, we propose repeating this experimental design in a few years to examine the effect of foliar fertilization on new and requested varieties on the market.

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Article

Oil-Based Polymer Coatings on CAN Fertilizer in Oilseed Rape (*Brassica napus* L.) Nutrition

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Abstract: Fertilizer coating can increase the efficiency of N fertilizers and reduce their negative impact on the environment. This may be achieved by the utilization of biodegradable natural coating materials instead of polyurethane-based polymers. The aim of this study was to detect the effect of calcium ammonium nitrate (CAN) fertilizer coated with modified conventional polyurethane enhanced with vegetable oils on the yield and quality of *Brassica napus* L. compared to CAN fertilizer with a vegetable oil-based polymer and to assess the risks of nitrogen loss. Three types of treatments were tested for both coated fertilizers: divided application (CAN, coated CAN), a single application of coated CAN, and a single application of CAN with coated CAN (1:2). A single application of coated CAN with both types of coating in the growth stage of the 9th true leaf significantly increased the yield, the thousand seed weight, and oil production compared to the uncoated CAN. The potential of using coated CAN may be seen in a slow nitrogen release ensuring the nitrogen demand for rapeseed plants throughout vegetation and eliminating the risk of its loss. The increased potential of NH_4^+ volatilization and NO_3^- leaching were determined using the uncoated CAN fertilizer compared to the coated variants. Oil-based polymer coatings on CAN fertilizer can be considered as an adequate replacement for partially modified conventional polyurethane.

Keywords: control release fertilizer; yield; oiliness; nitrogen losses; nitrate leaching

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1. Introduction

With the world's exponential population growth and diminishing of arable lands, the agriculture industry has faced a great challenge of crop and food resources for the past decades [1,2]. Predictions are that the earth's population could approach 9.5 billion by 2050, which may result in an almost double increase in food demand and crop production. In one specific example, cereal production is expected to increase from 940 million tons to 3 billion tons a year [3,4]. Satisfying increasing grain yield demands has been achieved by enhancing the use of mineral fertilizers to cropland soil. However, the excessive application of fertilizers presents one of the main sources of polluting soil (heavy metals), water (nitrates leaching into groundwater), and air environments (emission of greenhouse gases), which could be a threat to human health [5,6].

Nitrogen occupies a unique position among essential plant nutrients. Nitrogen and water availability are considered the two major limiting factors in plant growth and development of metabolic processes—nutrient distribution, photosynthesis, biomass, and ultimately yield building [7–9]. The deficiency of nitrogen strongly decreases chlorophyll content, enzymatic activity, photosynthesis, respiration rate, and yield of crops [10]. Nitrogen can be directly absorbed by plant roots in inorganic forms (mineral nitrogen) as ammonium (NH_4^+) and nitrate (NO_3^-). These forms are the key components of nitrogen fertilizers such as ammonium nitrate (AN) and urea included in the two most widespread nitrogen fertilizers [11].

According to FAO, world demand of nitrogen (N), phosphate (P_2O_5), and potash (K_2O) fertilizers were reported to be in total up to 184.0 mil tons in 2015. The forecast for 2022 could be up to 200.9 mil tons (nitrogen fertilizers present up to 111.6 mil tons) [12,13]. Despite the fact that the use of nitrogen fertilizers plays an essential role in meeting the demand for crop production, nitrogen use efficiency (NUE) is relatively low due to their excessive use (in general between 25–50%) and often leads to losses of redundant nitrogen from agroecosystems [14]. Nitrogen losses due to gaseous emissions of ammonia (NH_3), nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) along with leaching and runoff in the forms of ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) present an alarming threat to the environment [15].

Leakage prevention of nitrates may present one of the greatest environmental challenges in terms of nitrogen fertilizer use. Nitrogen losses, caused by NO_3^- leaching from soil into water, represent a loss of soil fertility and also pose a threat to the environment and subsequently to human health [5,16]. Increased nitrate levels present in drinking water create a risk of cancer, heart disease, and methemoglobinemia in babies [17]. According to calculations by Grizzetti et al. [17], up to 50% of the European population live in areas with a concentration of nitrates in water exceeding 25 $mg \cdot L^{-1}$, and up to 20% live in areas where nitrates exceed the recommended level of 50 $mg \cdot L^{-1}$. As already mentioned, nitrate coming from agriculture is the most common contaminant in the world's groundwater aquifers [18]. In the European Union, up to 38% of water bodies are significantly under pressure from agricultural pollution [19].

The application of controlled-release fertilizers (CRFs) is one way to improve nutrient use efficiency, reduce nitrogen loss, and contribute to minimizing environmental pollution, providing a better compromise among soil fertility, yield, and grain quality [20–22]. CRFs prove the potential to decrease the fertilizer application rate by 20% or 30% of the recommended value to achieve the same yield [23]. According to Trenkel [23] and Shaviv [24], CRFs can be defined as coated or encapsulated fertilizers by water-insoluble, semipermeable, or impermeable-with-pores materials, for which the factors determining the rate, pattern, and the duration of release have been known and regulated during the fabrication. Coating materials can be divided into two categories—inorganic materials (e.g., sulfur, bentonite, phosphogypsum) and organic polymers consisting of synthetic polymers derived from petroleum-based derivates (polyurethane, polyethylene, alkyd resin, etc.) or natural polymers (e.g., vegetable oil, starch, chitosan, cellulose) [23,25]. One of the most effective methods of preparing CRFs and thus the reduction of nutrient losses is by coating the surface of fertilizer with polyurethane materials. However, these coating materials are commonly linked with high costs and come from non-renewable petrochemical productions [26,27]. Furthermore, studies have shown that the residue of polyurethane shells in soils is difficult to degrade and may cause potential environmental risk [28]. This is one of the reasons why the agricultural industry has been searching for cheap, degradable, and renewable bio-based materials [29]. Vegetable oil is considered to be the most significant material for bio-based polymers, and polymeric material preparation to be an adequate substitution for polyurethanes [30]. Recent studies have shown that the use of oil-based polymers as coating materials led to gradual, uniform nutrient release and proved a high rate of biodegradability [31,32]. The most widely used vegetable oils to produce bio-based polymers are castor, linseed, canola, sunflower, palm, tobacco, corn, soy, and rapeseed [33].

The positive effect of CRFs in rapeseed cultivation has been described in several studies. The data show that the addition of coated N-fertilizers significantly increases the yield and quality of rapeseed [34,35]. Increased yield rates can be a consequence of advanced root volume and the improvement of plant biomass accumulation (especially during the growth stages of stem elongation, flowering, and harvest), extending the photosynthetic lifespan of pods [36–38].

The aim of our study was to evaluate the differences in the effectiveness of two newly developed coated fertilizers in nutritional status and yield of rapeseed and to assess their environmental impact (especially nitrate leaching). We assumed that the CAN fertilizer coated with a polymer-based on vegetable oil might provide comparable results with the same fertilizer coated with a modified conventional polyurethane.

2. Results and Discussion

The evaluation of the effect of coated fertilizers was created by comparing the data within the groups using the treatments with the same fertilizer application system (divided, single, and blends). Each method of fertilization was assigned with a control treatment (the treatments D and S). D served as the control variant for the group with a divided application, and S served as a control for the group with a single application and blends.

2.1. Yield and Oiliness of Rapeseed and N Content in Plant Biomass

The appropriate type of fertilizer and method of fertilization is important for the high yield production of rapeseed. Several studies describe the increase in yield and qualitative parameters of crops after using coated fertilizer application [38–41]. Our study showed that the use of coated CAN fertilizers has no negative effect on the yield and qualitative parameters of winter rapeseed. Statistical evaluation of the data shown in Figure 1 revealed no significant differences between the treatments in the groups with divided application (D, D-opu, D-o) and blends (S, Bl-opu, Bl-o). A significant positive effect was recorded in the group of treatments with a single application of coated CAN fertilizers (opu-CAN-oil-based polyurethane-coated CAN; o-CAN-oil-based polymer-coated CAN) in seed yields and oil contents. Seed yields of this group showed a trend of opu-CAN > o-CAN > CAN with opu-CAN up to 18% higher in comparison to the uncoated CAN. Similar results were recorded in the study by Tang et al. [42], in which a single basal application of coated nitrogen fertilizers contributed to the increase of the yield and rice quality in comparison to the divided application. A different trend was recorded in the case of the oil content that reached up to 5.5% higher after a single application of oil-coated CAN fertilizer compared to the use of the uncoated CAN fertilizer. The presumption was that the total nitrogen applied in the single application of coated fertilizers was released over a longer period of time and thus was present in the phase of the seed formation confirmed by Tian et al. [38]. In this study, the increase was recorded by an average of 17.3% after the application of coated fertilizers in rapeseed yield rates compared to the control. This study also proved that lower doses of the total N applied in coated fertilizers contributed to a yield increase of 14.2%, which confirmed their environmental potential in terms of nitrogen release. The study by Lu et al. [43] showed the positive effects of CRFs application on rapeseed yield manifested in the increase of rapeseed pods from 27 to 32% in comparison to non-coated urea. In comparison to the treatments with coated CAN fertilizers, a single application of the uncoated CAN (treatment S) proved the decline in the parameters of oil production and thousand seed weight (TSW) shown in Table 1. Similar positive effects of coated CAN fertilizers were proved on yield and qualitative parameters of rapeseed. It can be concluded that o-CAN may be a proper alternative instead of opu-CAN.

Table 1. Qualitative characteristics of rapeseeds.

Treatment	Oil Production (g/pot)	TSW (g)
D	8.4 ^{a} ± 0.8	30.2 ^{a} ± 2.2
D-opu	7.8 ^{a} ± 0.6	30.1 ^{a} ± 1.5
D-o	8.4 ^{a} ± 0.5	30.9 ^{a} ± 1.6
S	7.0 ^{b} ± 0.5	25.9 ^{b} ± 0.8
S-opu	8.7 ^{a} ± 0.5	30.6 ^{a} ± 1.4
S-o	8.8 ^{a} ± 0.6	30.5 ^{a} ± 1.6
S	7.0 ^{a} ± 0.5	25.9 ^{a} ± 0.8
Bl-opu	7.7 ^{a} ± 0.5	28.1 ^{a} ± 1.3
Bl-o	8.0 ^{a} ± 0.4	28.7 ^{a} ± 1.2

Groups of treatments D—divided application, S—single application; Bl—blend; opu—oil-based polyurethane polymer, o—oil-based polymer, TSW—thousand seed weight. The same letters next to the numbers depict no statistically significant differences between the treatments (Fisher's LSD test, $p \leq 0.05$). Each group of the treatment (divided, single, blend) was evaluated separately. The values represent the mean (n = 4) ± standard deviation (SD).

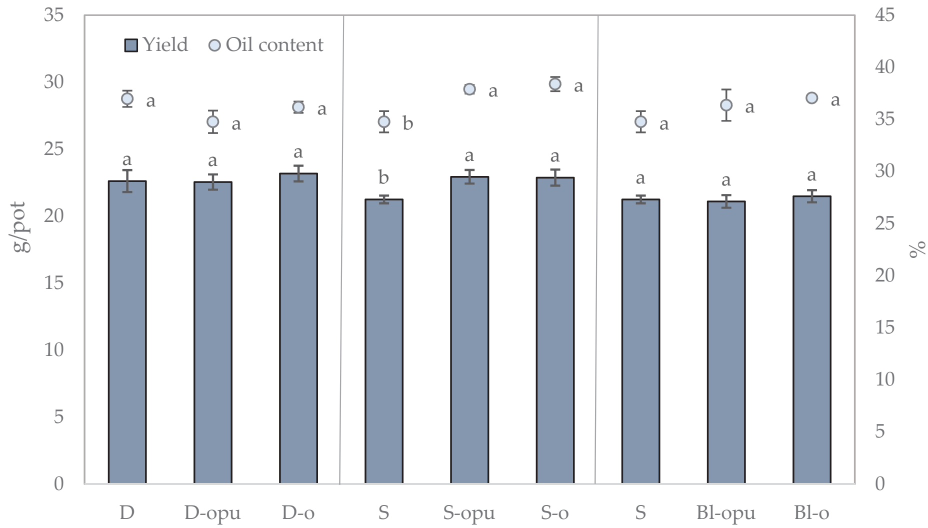

Figure 1. Rates of yield and oiliness of rapeseed. The groups of the treatments D—divided application; S—single application; Bl—blend. The columns represent the mean (n = 4), error bars present the mean standard deviation (SD). The same letters at the top of the columns describe no statistically significant differences between the treatments (Fisher's LSD test, $p \leq 0.05$). Each group of the treatment (D, S, Bl) was evaluated separately.

The data from Figure 2 indicate a connection between the yield rates and the nitrogen concentration in aboveground plant biomass. In general, plants can only consume a part of nutrients (in our case nitrogen) from conventional fertilizers, and the rest may be subject to losses to the environment [44]. This trend is mainly visible in the treatment with the application of conventional uncoated CAN fertilizer in a single dose (S), resulting in a significantly lower concentration of nitrogen in plant biomass in the growth stage of flower bud emergence (*t2*) compared to the growth stage of stem elongation (*t1*). This decrease indicates that the overdose of quickly released nitrogen in uncoated CAN fertilizer led to N-loss available for direct plant consumption and ultimately caused the lowest yield and oil content. The declining trend in the supply of the available form of N, released from conventional uncoated CAN, during the period and the increased supply of mineral N released from coated CAN is also evident from the assessment of N content in aboveground biomass (Table 2). The nitrogen content in the plant shows a gradual release of the available forms of this nutrient from the coated CAN that is particularly evident in the group of singly applied fertilizers (S). While the nitrogen content detected in the aboveground mass of rapeseed fertilized with uncoated CAN (S) was detected almost 4 and 2 times higher in the term *t1* compared to the treatments with coated CAN (S-opu, S-o) in the term *t2*, the nitrogen content of the treatments fertilized with coated fertilizers was increased. These values show that the oil-coated CAN is able to release nitrogen more rapidly than the oil-based, polyurethane-coated CAN and thus may supply the plant's demand for this nutrient. Nitrogen contents in plants, treated with coated fertilizers applied in blends with conventional CAN (Bl-opu, Bl-o), can confirm this trend.

The relationship between the optimal nitrogen supply and its impact on the yield and oil content of rapeseed is described in many studies [45,46]. A similar trend was recorded in the treatments with coated CAN fertilizers applied in blends with the uncoated CAN fertilizer (Bl-opu, Bl-o). Nitrogen content in plant biomass in the growth stage of stem elongation decreased about 1.3% and 0.9% compared to the uncoated CAN fertilizer applied in a single dose. The N content in plant rapeseed showed the most even N pumping during vegetation in the variant with divided application and a single application of coated CAN fertilizers.

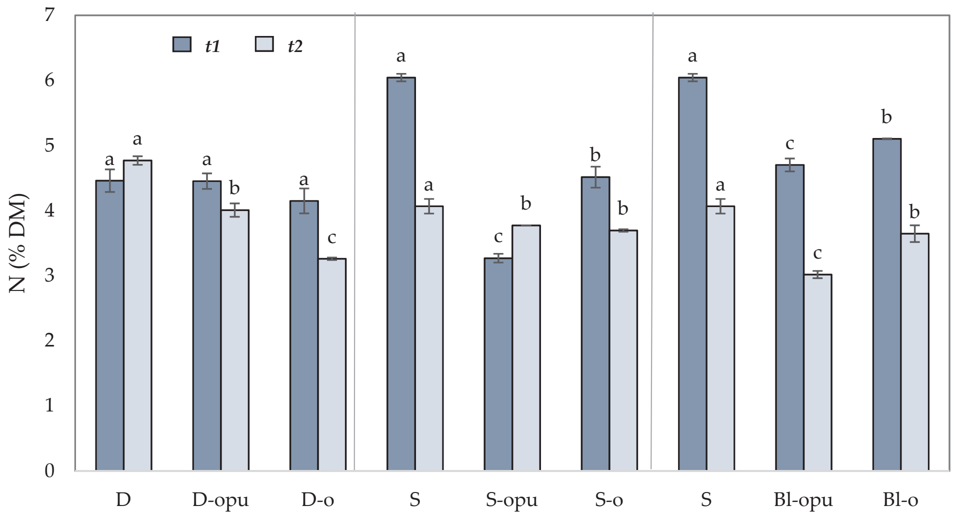

Figure 2. Nitrogen concentration (% DM) in aboveground plants dry matter collected in two growth stages *t1* and *t2* of rapeseed. The groups of treatments D—divided application, S—single application; Bl—blend; opu—oil-based polyurethane polymer, o—oil-based polymer. The columns represent the mean (*n* = 4), error bars present the mean standard deviation (SD). The same letters at the top of the columns describe no statistically significant differences between the treatments (Fisher's LSD test, $p \leq 0.05$). Each group of the treatment (D, S, Bl) was evaluated separately.

Table 2. Content of nitrogen in aboveground plant dry matter (mg/plant).

Treatment	Nitrogen Content (mg/plant)	
	t1	*t2*
D	68.3 ^{a} ± 0.9	187.4 ^{a} ± 29.2
D-opu	69.6 ^{a} ± 1.4	155.0 ^{ab} ± 2.2
D-o	64.1 ^{b} ± 2.8	123.5 ^{b} ± 8.0
S	135.0 ^{a} ± 1.3	215.6 ^{a} ± 23.9
S-opu	36.9 ^{c} ± 0.2	112.0 ^{c} ± 15.8
S-o	68.6 ^{b} ± 1.6	174.6 ^{b} ± 0.3
S	135.0 ^{a} ± 1.3	215.6 ^{a} ± 23.9
Bl-opu	86.3 ^{c} ± 1.2	135.6 ^{b} ± 3.8
Bl-o	97.4 ^{b} ± 2.8	183.6 ^{a} ± 16.1

Groups of treatments D—divided application, S—single application; Bl—blend; opu—oil-based polyurethane polymer, o—oil-based polymer. The same letters next to the numbers depict no statistically significant differences between the treatments (Fisher's LSD test, $p \leq 0.05$). Each group of the treatment (divided, single, blend) was evaluated separately. The values represent the mean (*n* = 4) ± standard deviation (SD).

2.2. Mineral Nitrogen Content in the Soil

The release of nitrogen from coated CAN fertilizers significantly affected the dynamic change of the soil mineral N (N_{min}) content in the growth process of rapeseed. Contents of N_{min} and its ionic forms (NO_3^-, NH_4^+) were determined in the soil in three experimental phases (*t1*–*t3*,). Although, enough of the available nitrogen can be essential for direct plant consumption. The excessive content may inevitably increase its loss in soil [47]. Average contents of N_{min} in soil (without differencing into layers), shown in Table 3, serve as an overview of nitrogen release development in the treatments during the rapeseed vegetation.

Table 3. Contents of mineral nitrogen (N_{min}) in soil (mg/kg) on selected experimental phases (*t1*–*t3*).

Treatment	*t1*	*t2*	*t3*
D	$18.64^{b} \pm 1.34$	$62.84^{b} \pm 1.27$	$10.69^{a} \pm 1.04$
D-opu	$15.31^{a} \pm 1.22$	$20.34^{a} \pm 3.53$	$10.51^{a} \pm 0.03$
D-o	$14.23^{a} \pm 1.72$	$23.33^{a} \pm 1.25$	$18.21^{b} \pm 3.09$
S	$108.42^{c} \pm 3.37$	$37.54^{c} \pm 6.44$	$8.54^{a} \pm 0.60$
S-opu	$20.07^{a} \pm 0.56$	$11.62^{a} \pm 0.56$	$13.27^{b} \pm 2.34$
S-o	$35.33^{b} \pm 2.89$	$19.42^{b} \pm 1.22$	$19.17^{c} \pm 2.90$
S	$108.42^{c} \pm 3.37$	$37.54^{b} \pm 6.44$	$8.54^{a} \pm 0.60$
Bl-opu	$37.51^{a} \pm 6.01$	$18.05^{a} \pm 3.02$	$11.31^{b} \pm 1.17$
Bl-o	$50.73^{b} \pm 2.22$	$24.34^{ab} \pm 5.09$	$11.99^{b} \pm 0.99$

Groups of treatments D—divided application, S—single application; Bl—blend; opu—oil-based polyurethane polymer, o—oil-based polymer. The same letters next to the numbers describe no statistically significant differences between the treatments (Fisher's LSD test, $p \leq 0.05$). Each group of the treatment (divided, single, blend) was evaluated separately. The values represent the mean (n = 4) ± standard deviation (SD).

One of the important aspects of coated fertilizers is the longevity of nutrient release in sufficient levels for plant uptake. The use of coated CAN fertilizers in each form of the application (D, S, and Bl) has shown a positive effect on N_{min} release pattern, as can be seen from Figure 3. The effect was visible, especially in the period between the first (*t1*) and the second term (*t2*) of soil samples collection that was significantly milder compared to conventional uncoated CAN.

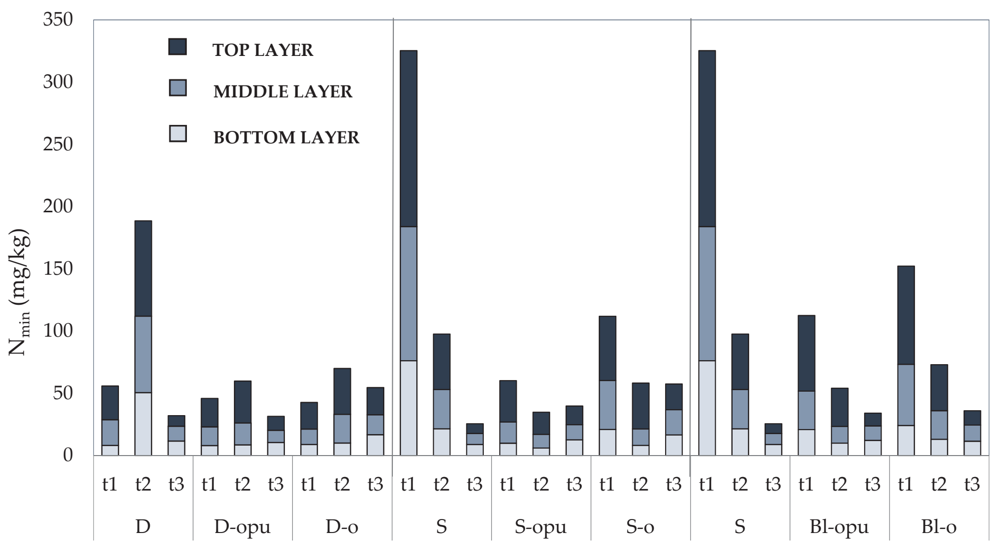

Figure 3. Contents of mineral nitrogen (N_{min}) in soil (top, middle and bottom layer of pot) in three experimental phases (*t1, t2, t3*). Groups of treatments D—divided application, S—single application; Bl—blend; opu—oil-based polyurethane polymer, o—oil-based polymer. The columns represent the mean (n = 4).

The relatively accelerated release of nitrogen was observed in high N_{min} concentration after the application of fertilizers (*t1* single application, *t2* divided application) in the treatments with conventional uncoated CAN shown in Table 3. Rapid release N_{min} was visible mainly in the single application in which N_{min} concentration decreased rapidly up to 65.4% between *t1* and *t2* (up to 22 days). Our assumption was that although the part of the soil N_{min} was obtained from the soil through plant roots, the great contrast in N_{min} concentration was due to N loss (NH_4^+ volatilization and NO_3^- leaching) between *t1* and *t2*. On the contrary, the data of the soil samples, collected in the harvest time (*t3*), showed relatively high levels of N_{min} in the treatments with divided (especially D-o) and single (S-opu, S-o) application of fertilizers in comparison with conventional CAN treatments. Dynamic of gradual N_{min} release was most visible after a single application of both coated CAN (S-opu, S-o) with no definite decrease in N_{min} content in *t3*. A single application of oil-based polyurethane-coated CAN fertilizer (S-opu) caused an increase by 14.2% in *t3* in mineral nitrogen content compared to *t2* in soil. These findings corresponded to the data of yield and qualitative parameters (Figure 1), in which a single application of coated CAN fertilizer (S-opu) proved to be the most effective. The assumption was that the amount of released nitrogen reached sufficient levels for the plant demand in the time of the experiment duration from these treatments, thus leading to the increased nitrogen use efficiency and subsequently to a more positive environmental impact (lower risk of N loss). Our data are consistent with the findings of Xiao et al. [48], who described that the total N_{min} content continued gradually to an increase in the top layer of soil on the ninetieth day after the application of coated fertilizers, while high levels were maintained in the middle and bottom layer of soil.

The positive effect of coated CAN fertilizers on N_{min} content was also visible in the nitrogen distribution between soil layers during the experiment (Figure 3). The application of conventional uncoated CAN fertilizer (D and S treatment) showed high N_{min} concentrations mainly in the top and middle layers of the soil right after fertilization. The treatments with coated CAN fertilizers showed that N_{min} content was, in general, focused mainly on the top layer of the soil during *t1* and *t2*. N_{min} content was evenly distributed between each layer of the soil in the harvest time (*t3*). This indicates that both coated CAN fertilizers (opu-CAN and o-CAN) proved a high ability of gradual nitrogen release leading to more efficient nitrogen use by the plant and a reduction in the environmental risk. A gradual N_{min} release by coated fertilizers was also described in the study by Zheng et al. [49], who found that the application of coated fertilizers resulted in enhanced N_{min} concentration in soil, especially during later crop stages.

Considering the placement of the fertilizers (the placement on the soil surface without incorporation to the soil), the highest potential for the NH_4^+ volatilization is most likely to be closest to the soil surface [50]. Ammonium nitrate (used CAN in our experiment), depending on N dose and irrigation, belongs to the conventional nitrogen fertilizers with a high potential of NH_4^+ volatilization [51].

This assumption was confirmed by the data obtained from the top layer of the soil samples (Figure 4). The data showed the greatest potential for NH_4^+ volatilization in the treatments with conventional uncoated CAN (D and S treatments) expressed in significantly high NH_4^+ concentrations in *t1* and *t2*. Analogous to N_{min}, the uncoated CAN potential of volatilization was visible between *t1* and *t2*, in which the NH_4^+ concentration decreased up to 39.8% in soil. Higher NH_4^+ concentrations were accountable to the use of conventional uncoated CAN (1/3 of the total N dose) after the application of blend fertilizers (Bl-opu, Bl-o). Similarly, the S variant (a single application of uncoated CAN) was resolved in its rapid release. NH_4^+ contents in Bl-opu and Bl-o were detected almost over half lower in *t1* than in the S treatment; therefore, major risks of NH_4^+ losses were not found. In addition to the volatilization, a rapid NH_4^+ release also presents the risk of the increased concentration of nitrates as an initial component of nitrification in soil and thus increased the risk of NO_3^- leaching [52].

The positive effect of coated fertilizers was expressed by significantly lower NH_4^+ concentrations during *t1*–*t3* in comparison to conventional uncoated CAN. The data were indirectly consistent with the findings of Xiao et al. [48], who mentioned that the application of coated fertilizers resulted in lower NH_4^+ rates in soil samples in comparison to conventional uncoated nitrogen fertilizer. A gradual NH_4^+ release was also expressed by the increase of NH_4^+ concentration in the top layer of the soil in *t2*. This fact was noticeable in the treatments of D-opu (up to 41.3%), D-o (up to 58.8%), and S-o (up to 29.7%). The treatments with a divided and single application of fertilizers were proved to be the most efficient in terms of the longevity of NH_4^+ release. These types of fertilizer applications showed significantly higher NH_4^+ contents in *t3* treatments compared to the treatments with conventional uncoated CAN. On the contrary, NH_4^+ contents showed no significant difference in the S treatment in Bl-opu and Bl-o. This led to an assumption that all nitrogen contained in coated fertilizers and applied in blends was released during the rapeseed vegetation, predetermining the blend application as the most suitable alternative.

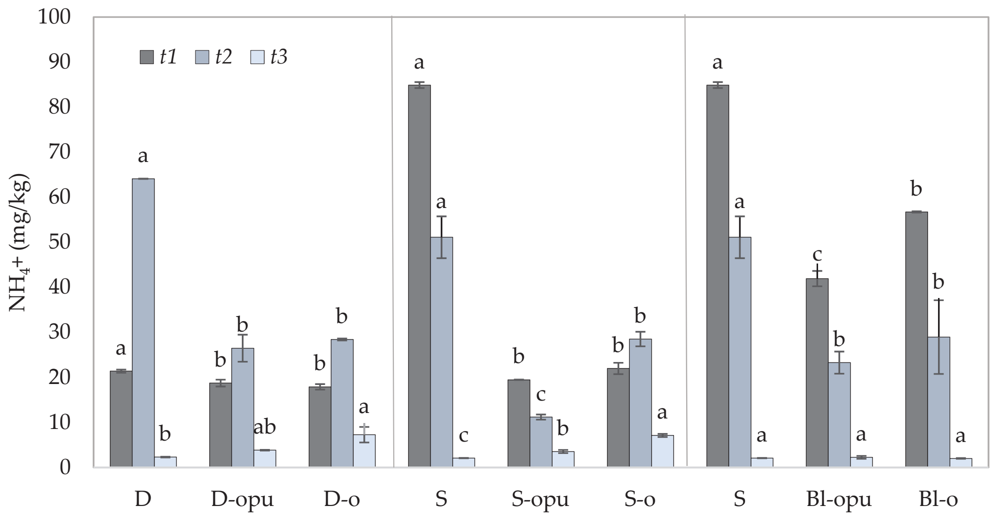

Figure 4. Contents of NH_4^+ in the top layer of the soil samples collected in three experimental phases (*t1*–*t3*). Groups of treatments D—divided application, S—single application; Bl—blend; opu—oil-based polyurethane polymer, o—oil-based polymer. The columns represent the mean ($n = 4$), error bars present the mean standard deviation (SD). The same letters at the top of the columns depict no statistically significant differences between the treatments (Fisher's LSD test, $p \leq 0.05$). Each group of the treatment (D, S, Bl) was evaluated separately.

Contents of NO_3^- were monitored as the main potential source of N loss in the soil samples due to their high leaching ability. One of the first studies by Liegel and Walsh from 1976 [53] proved that the application of controlled-release N fertilizers was the most effective technique in sandy irrigated soils with a high risk of nitrate leaching. Preventing the leaching of nitrates presents one of the greatest environmental challenges in terms of nitrogen fertilizer use. The estimation of the potential for N losses due to the NO_3^- leaching from the experimental treatments were provided by the isolation of the data from the bottom and middle layers of soil. The data obtained from the middle layer (ML) of the soil (Figure 5) served for the evaluation of potential NO_3^- migration to the lower layers of the soil, which might consequently lead to its leaching into the groundwater. The data obtained from the bottom layer (BL) of the soil (Figure 6) served to evaluate the potential of nitrates leaching to the groundwater during the rapeseed vegetation and directly after its harvest.

As predicted, significantly, the highest potential for NO_3^- leaching was due to rapid nitrogen release from conventional uncoated CAN fertilizers recorded in single or divided CAN application. The potential for NO_3^- leaching after uncoated CAN application was possible to confirm from the data of NO_3^- concentrations in *t1* and *t2* shown in Figures 5 and 6. The NO_3^- content of ML and BL was detected over three times higher (>3.3) in the treatment fertilized with a single application of uncoated CAN in *t1* compared to the treatments with coated CAN fertilizers. The data showed that the NO_3^- decrease was found up to 73.9% in ML and up to 75.5% in BL in the S treatment between *t1* and *t2*. Considering the amount and duration (up to 14 days), it is most likely that nitrates of the uncoated CAN fertilizer were lost due to the nitrate leaching. These findings corresponded with the data by Zhang et al. [54], who discovered that the rates of the leached nitrates in water samples were detected significantly higher in comparison to coated urea in the treatments with conventional urea.

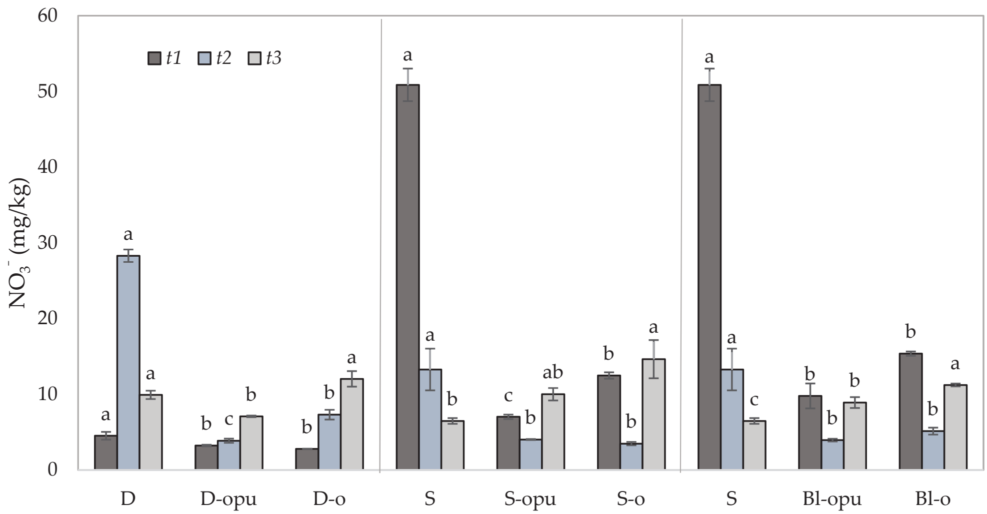

Figure 5. Contents of NO_3^- in the middle layer of soil samples collected in three experimental phases (*t1*–*t3*). Groups of treatments D—divided application, S—single application; Bl—blend; opu—oil-based polyurethane polymer, o—oil-based polymer. The columns represent the mean (n = 4), error bars present the mean standard deviation (SD). The same letters at the top of the columns depict no statistically significant differences between the treatments (Fisher's LSD test, $p \leq 0.05$). Each group of the treatment (D, S, Bl) was evaluated separately.

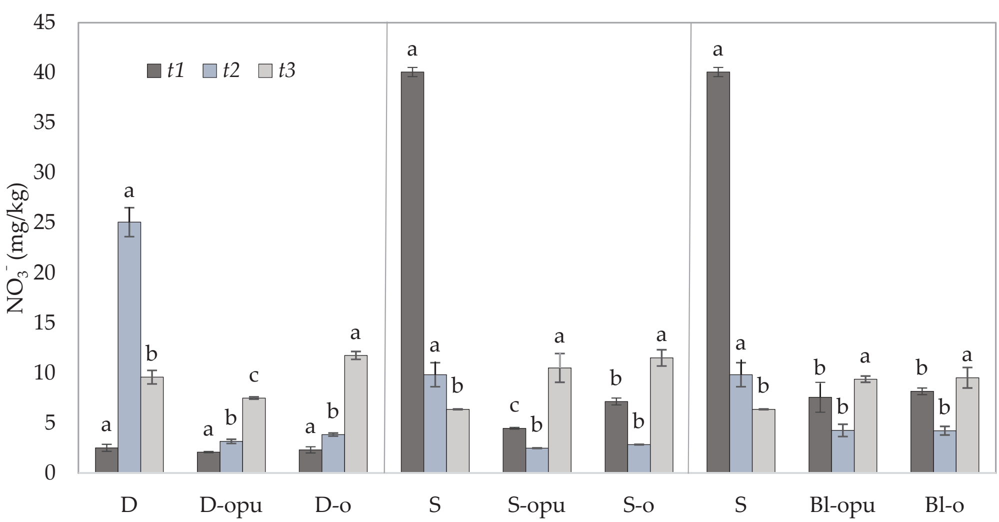

Figure 6. Contents of NO_3^- in the bottom layer of soil samples collected in three experimental phases (*t1*–*t3*). Groups of treatments D—divided application, S—single application; Bl—blend; opu—oil-based polyurethane polymer, o—oil-based polymer. The columns represent the mean (n = 4), error bars present the mean standard deviation (SD). The same letters at the top of the columns depict no statistically significant differences between the treatments (Fisher's LSD test, $p \leq 0.05$). Each group of the treatment (D, S, Bl) was evaluated separately.

Identical to N_{min} and NH_4^+, the positive effect of coated CAN fertilizers was recorded in the form of gradual NO_3^- release over the course of the whole experiment. Gradual release of nitrates was discovered to be the most visible between *t2* and *t3* in coated fertilizers. The increased NO_3^- contents were observed up to 64.7% in ML and up to 119.9% in BL. While the NO_3^- amount was decreased in ML and BL in the treatments fertilized with uncoated CAN, the coated CAN fertilizers were able to supply the plants with nitrogen even in the later stages of the development. Compared to the low levels of NO_3^- content in the treatments with conventional CAN fertilizers (due to rapid nitrogen release and subsequent N loss). This increase correlated with the data of seed yield and qualitative parameters (Figure 1) and can be used as a potential supply of available nitrogen for the next crops. The data correlated with the findings of Xiao et al. [48]. Similar N_{min} release (especially NO_3^-) was proved using oil-based polymer-coated CAN, which can be a proper alternative for oil-based polyurethane-coated CAN. This fact is not suitable for future use due to polyurethane's lower biodegradability. The positive effect of coated fertilizers on nitrates leaching was recorded in several studies [55–58].

3. Materials and Methods

The pot experiment was performed under controlled conditions in the vegetation hall of Mendel University in Brno, Brno, Czech Republic (49°12′36.94″ N and 16°36′49.95″ E).

3.1. Plant Material and Growth Conditions

Rapeseed (*Brassica napus* subs. *napus*) cv. DK Exception (Bayer s.r.o, Prague, Czech Republic) was used in this study. Mitscherlich pots (STOMA GmbH, Siegburg, Germany) were filled with 6 kg of air-dried and <2 cm sieved soil and placed in the vegetation hall. The properties of the used soil for the pot experiment are shown in Table 4. Ten seeds of rapeseed were sown in 2 cm depth in each pot. Three weeks after sowing, the number of rapeseed plants was adjusted to three plants per pot.

Table 4. Agrochemical properties of used soil.

Soil Parameter	Value	Devices	Ref.
Soil type	Stagnic Fluvisols (FL-st)		[59]
Clay	53%	Pipette apparatus (NEN 5753:2018)	[60]
pH ($CaCl_2$)	6.6	pH meter, inoLab pH/ION Level 2 with SenTix 62 pH electrode	[61]
Soil electrical conductivity (EC)	0.05 mS/cm	EC meter, inolab pH/ION Level 2 with WTW TetraCon 325	[61]
Soil oxidizable carbon (C_{ox})	1.28%	Walkley-Black method	[62]
NH_4^+ (K_2SO_4)	1.48 mg/kg	UV/VIS Spectrometer, Unicam 8625	[61]
NO_3^- (K_2SO_4)	17.2 mg/kg	NO_3^--ISE	[61]
P (Mehlich III)	201 mg/kg	UV/VIS Spectrometer, Unicam 8625	[61]
K (Mehlich III)	367 mg/kg	AAS, ContrAA 700	[61]
Ca (Mehlich III)	3015 mg/kg	AAS, ContrAA 700	[61]
Mg (Mehlich III)	294 mg/kg	AAS, ContrAA 700	[61]
S (water-soluble)	13.8 mg/kg	ICP-MS, Agilent 7900	[61]

Mehlich III—soil test extractant.

3.2. Experimental Design

In the experiment, coated CAN fertilizers were compared with a conventional uncoated CAN. The same total dose of nitrogen was applied in all treatments using different N sources such as calcium ammonium nitrate (CAN, up to 13% N-NH_4^+ and 13% N-NO_3^-, Lovochemie a.s., Lovosice, the Czech Republic), oil-based polyurethane-coated CAN (opu-CAN) and oil-based polymer-coated CAN (o-CAN). Coated fertilizers were prepared by spreading the coating on conventional fertilizer CAN using the LDP-3 fluidized bed granulating machine (Changzhou Jiafa Granulating Drying Equipment Co., Ltd., Changzhou, China). The coating consisted of oil-based polyurethane polymer (opu-CAN—coating up to 7.6 wt.%, up to 13% N-NH_4^+ and 13% N-NO_3^-, VUCHT a.s., Bratislava, Slovakia) and oil-based polymer (o-CAN—coating up to 6.1 wt.%—triglycerides of fatty acids, up to 75 wt.% of which unsaturated were up to 45 wt.%, polylactic acid up to 10 wt.%, up to 13% N-NH_4^+ and 13% N-NO_3^-, VUCHT a.s., Bratislava, Slovakia). The composition of the polyurethane-based coating (opu-CAN) differed from the conventional polyurethanes prepared by the reaction of the diisocyanates with the polymeric diols. The polymeric diols were replaced with the vegetable oil having hydroxy groups in its structure. The prepolymer, obtained by this way, was finally applied in the crosslinking. These modifications led to a substantial increase in the biodegradable fraction of the coating. The prepolymer was completely replaced with a more biodegradable component in the oil-based coating (o-CAN). The biodegradable fraction of the coating material is further increased in this way.

The individual treatments and fertilizer addition are detailed in Table 5. The fertilizers were applied to the soil surface. Each treatment was replicated 8 times in a complete randomized block design in the vegetation hall (Figure 7).

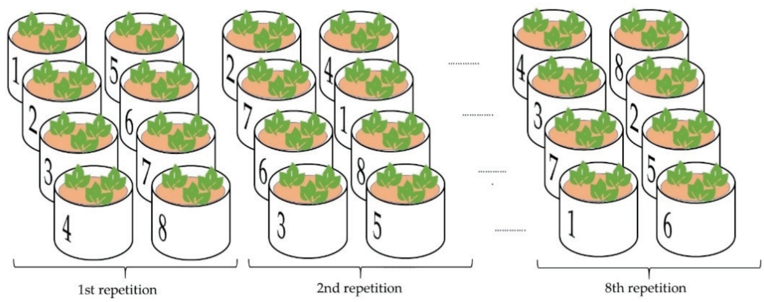

Figure 7. Schematic illustration of the layout of the pots (and their repetition) in the vegetation experiment. Treatment D (1), D-opu (2), D-o (3), S (4), S-opu (5), S-o (6), Bl-opu (7), Bl-o (8).

The treatments of fertilizer were divided into 3 groups according to the term of application and the type of fertilizer chosen. The first group was the divided application of fertilizers (the designation of the treatments with D). The total nitrogen dose was divided into two parts; the first was applied by the conventional uncoated CAN in the 1st term (1st Fertilization), the second dose was applied by uncoated CAN (treatment D) and coated CAN (treatments D-opu and D-o) in 2nd term (2nd Fertilization). The second and third groups consisted of treatments with a single application of total nitrogen dose in one term (1st Fertilization), where fertilizers of one type (the designation of the treatments with S) and fertilizers of a mixture (the designation of the treatments with Bl) were applied. The fertilizer mixtures (Bl) were created by mixing conventional CAN and coated CAN in a 1:2 ratio (converted to N rate).

The pot experiment was carried out under semi-natural conditions (under a rain shelter) in the vegetative hall. Figure 8 shows the average daily temperature and the average daily relative humidity during the experiment. A controlled watering regime, used identical for all treatments (pots), was in the experiment. Plants were watered to 70% of maximum water holding capacity throughout the growing season. The pots were hand-watered with demineralized water on the soil surface.

Rapeseed plants were harvested manually by cutting above the soil surface from each pot. The rapeseed was threshed using a laboratory thresher (HALDRUP LT-20, Haldrup GmbH, Ilshofen, Germany).

The rape seeds were purified from coarse impurities by repetitive sifting. Rapeseed yield was measured in three plants within each pot, and the value was adjusted to 9% of moisture. Seed yield was determined by weighing (laboratory scale PCB Kern, KERN & Sohn GmbH, Balingen, Germany) and exceeded as gram per pot (g/pot). Seeds were then counted and hand-ground in mortar for further analysis of the oil content.

Table 5. Design of treatments and nitrogen applications.

Treatment	Fertilizer	Application/Ratio	Dose of N in g per Pot (mg/kg Soil)		
			1st Fertilization	2nd Fertilization	Total Dose of N
D	CAN + CAN	divided (1:2)	0.408 (68)	0.848 (141)	1.256 (209)
D-opu	CAN + opu-CAN	divided (1:2)	0.408 (68)	0.848 (141)	1.256 (209)
D-o	CAN + o-CAN	divided (1:2)	0.408 (68)	0.848 (141)	1.256 (209)
S	CAN	single	1.256 (209)	-	1.256 (209)
S-opu	opu-CAN	single	1.256 (209)	-	1.256 (209)
S-o	o-CAN	single	1.256 (209)	-	1.256 (209)
Bl-opu	CAN + opu-CAN	single (blend 1:2)	0.408 + 0.848 (68 + 141)	-	1.256 (209)
Bl-o	CAN + o-CAN	single (blend 1:2)	0.408 + 0.848 (68 + 141)	-	1.256 (209)

Groups of treatments D—divided application, S—single application; Bl—blend; opu—oil-based polyurethane polymer, o—oil-based polymer.

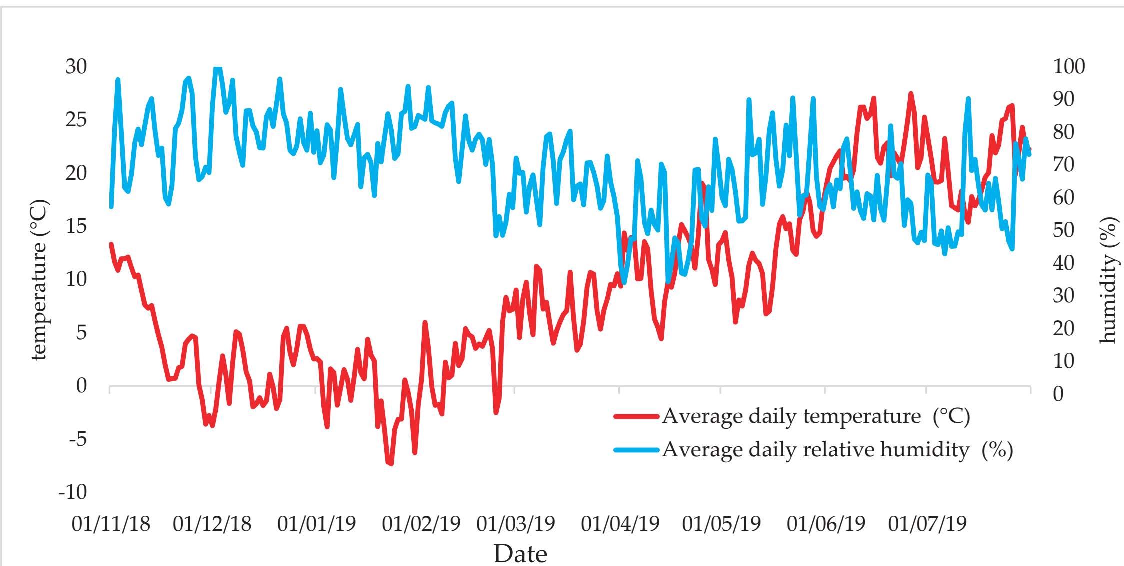

Figure 8. The average daily temperature (°C) and relative humidity (%) in the vegetation hall during the experiment.

3.3. Plants and Soil Sampling

The evaluation of soil mineral nitrogen content (NO_3^-, NH_4^+) and nutritional plant properties was provided in the soil samples and plant biomass collected in the specific experimental phases shown in Table 6. The collection of the soil samples was carried out by a probe with the aligned tip. After the collection, the soil profile was divided in three zones for the observation of mineral nitrogen movement in soil and subsequently frozen for further analysis (Figure 9).

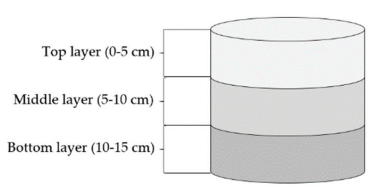

Figure 9. Illustration of soil layers layout in the pot used to determine N_{min} content.

The plant biomass was dried at 50 °C and homogenized to determine the nitrogen content in the dry matter.

Table 6. Experimental phases and dates.

Experimental Phases	Date	Rape Growth Stages	Term
Start of the experiment (sowing)	1 November 2018	seed Dry	
1st Fertilization	11 March 2019	nine or more leaves unfolded	
1st Plant and soil sampling 2nd Fertilization	2 April 2019	stem elongation	*t1*
2nd Plant and soil sampling	16 April 2019	flower bud emergence	*t2*
3rd Soil sampling; harvest	16 July 2019	harvested product	*t3*

3.4. Analytical Methods

The N_{min} determination was provided according to the methodology by Zbíral et al. [62], who described that nitrate and ammonium nitrogen was extracted from the soils with a solution of neutral salt (1% of K_2SO_4). The NH_4^+ determination was carried out spectrophotometrically ($\lambda_{660\ nm}$). The NO_3^- contents were determined by ISE (Ion Selective Electrode) [63].

The nitrogen determination was provided in aboveground plant biomass according to the methodology by Zbíral et al. [64]. Nitrogen contents were determined by the Kjeldahl method using the Kjeltec 2300 device (Foss, Hillerød, Denmark).

The thousand seed weight (TSW) determination was performed using a laboratory counter MK (MEZOS spol. s r.o., Hradec Králové, the Czech Republic). The determination was carried out by weighing the number of 2 × 500 seeds to prevent possible measurement errors.

The determination of seed oil content was provided according to the methodology of the Central Institute for Supervising and Testing in Agriculture [65]. The oil content was determined gravimetrically after the extraction of the samples with diethyl ether using the Soxhlet method based on the NMR extraction of rapeseeds in a continuous flow extractor Minispec mq series TD-NMR (Bruker Corporation, Ettlinger, Germany).

3.5. Statistical Analysis

The effect of the treatment on the evaluated parameters was statistically analyzed in the STATISTICA 12 program (TIBCO Software, San Jose, CA, USA) [66]. The effect of the treatment on the seed yield, oiliness, oil production, thousand seed weight, nitrogen concentration and content in aboveground plant biomass and the content of mineral nitrogen (ammonium and nitrate) in soil were analyzed separately for each group of the treatment (divided, single and blend application of fertilizers). The normality and homogeneity of variances were verified, respectively, by Shapiro-Wilk and Levene values at $p \leq 0.05$. The influence of the monitored factors was analyzed via analysis of variance (level of significance $p \leq 0.05$). The effect of the treatment on the mentioned parameters was analyzed using two-way analyses of variance with the treatment such as fixed effect and the pot used as the random effect to take into account the grouping of individuals in the same pot. The differences between the means were evaluated by the Fisher's (*LSD*) test.

4. Conclusions

The use of coated CAN fertilizer proves the potential to gradually release acceptable nitrogen during the growing season in winter rape nutrition and thus continuously meet the needs of plants. Compared to the effect of conventional CAN, the use of coated CAN fertilizers has been shown to increase the efficiency of nitrogen fertilization and reduce its losses. A suitable method seems to be the application of a mixture of conventional CAN and coated CAN in a ratio of 1:2 during spring fertilization, ensuring a sufficient amount of rapidly releasing N during the regeneration of rapeseed and its slower release during further developmental stages. The CAN fertilizer coated with a biodegradable oil-based polymer proves the ability to release the optimum amount of nitrogen for canola nutrition.

The use does not pose a risk of rapid release of mineral N in quantities potentially polluting the atmosphere (ammonia volatilization) and hydrosphere (nitrate leaching). According to these results, the CAN fertilizers coated with a polymer-based on vegetable oils could be used as a replacement for commonly used synthetic polymers based on polyurethane confirming the initial hypothesis.

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Technologies for Fertilizers and Management Strategies of N-Fertilization in Coffee Cropping Systems to Reduce Ammonia Losses by Volatilization

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Abstract: The aim of this study was to quantify NH_3-N losses from conventional, stabilized, slow-release, and controlled-release N fertilizers in a coffee field. The N fertilizers analyzed were prilled urea, prilled urea dissolved in water, ammonium sulfate (AS), ammonium nitrate (AN), urea + Cu + B, urea + adhesive + $CaCO_3$, and urea + NBPT (all with three split applications), as well as blended N fertilizer, urea + elastic resin, urea-formaldehyde, and urea + polyurethane (all applied only once). NH_3-N losses (mean of two crop seasons) were statistically higher for urea + adhesive + $CaCO_3$ (27.9% of applied N) in comparison with the other treatments. Loss from prilled urea (23.7%) was less than from urea + adhesive + $CaCO_3$. Losses from urea + NBPT (14.5%) and urea + Cu + B (13.5%) were similar and lower than those from prilled urea. Urea dissolved in water (4.2%) had even lower losses than those treatments, and the lowest losses were observed for AS (0.6%) and AN (0.5%). For the single application fertilizers, higher losses occurred for urea + elastic resin (5.8%), blended N fertilizer (5.5%), and urea + polyurethane (5.2%); and urea-formaldehyde had a lower loss (0.5%). Except for urea + adhesive + $CaCO_3$, all N-fertilizer technologies reduced NH_3-N losses compared to prilled urea.

Keywords: N-fertilizers; NH_3 emission; urease inhibitors; slow- and controlled-release N-fertilizers; *Coffea arabica*; sustainable agriculture

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1. Introduction

Brazil is the largest coffee (*Coffea arabica* L.) producer and exporter worldwide, and the constant search for better beverage quality and sustainability is essential in different coffee production systems. The application of nitrogen (N) fertilizers is imperative to achieve an adequate yield of this high-value crop. Nitrogen is the nutrient most extracted by the coffee plant and the nutrient of second greatest export by coffee beans [1,2].

Some studies using ^{15}N have shown that coffee plants take up less than 25% of the N fertilizer when applied as conventional urea [3,4]. The dynamic transformation of N forms in the soil and the varying pathways of N losses in coffee growing areas result in low N fertilizer use efficiency (NUE) [4]. Ammonia (NH_3-N) volatilization is the primary N loss in coffee production areas in Brazil [2,5,6], particularly when conventional urea is applied on the soil surface with plant residues and without fertilizer incorporation [7]. This is a common practice in systems of perennial crops such as coffee.

In 2017, the amount of N fertilizers used in the world was estimated at 150 Tg N per year [8], and may reach 260 Tg N per year in 2050 [9]. About 50% of global N fertilizer production is represented by urea [10,11]. The NH_3-N losses from urea can be intensified when specific soil properties are combined with climatic conditions favorable to this loss. Such properties and conditions include the application of urea on moist soil followed by an absence of rainfall, increased soil and air temperature, increased N doses, application of N on soils with low cation exchange capacity, and alkaline soil pH [12,13]. NH_3-N losses can exceed 50% of the applied dose, considering N applications in multiple crops [14,15]. The equivalent of one in three applications of N fertilizer is lost by volatilization in coffee production systems using conventional urea as an N source [2,5,16].

NH_3-N losses not only reduce NUE and cause agronomic damage, but also lead to environmental problems. These problems include air pollution, due to the acidifying nature of NH_3 [17], and greenhouse gas emissions to the atmosphere. NH_3 gas is an indirect source of nitrous oxide (N_2O), which has a global warming potential 310 times greater than carbon dioxide (CO_2) [18]. It is estimated that 1.4% of the total volatilized N is converted to or lost as N_2O [19].

The 4R principles (right nutrient source, right rate, right time, and right place) guide various management practices to minimize nutrient losses and the C footprint and increase N retention in the soil [20]. The development and proper use of enhanced-efficiency fertilizers (EEFs) may reduce these N losses [20–22]. Technological development of fertilizers is currently one of the strategies most investigated for improving NUE [23–25].

The fertilizer technology market had a compound annual growth rate estimated at 12% from 2014 to 2020 [26]. In addition, some European countries, including Germany and the Netherlands, have already adopted measures banning the application of conventional urea without incorporation and encouraging the use of some technologically enhanced fertilizer.

Enhanced efficiency fertilizers are in four main categories: stabilized fertilizers, slow-release fertilizers, controlled-release fertilizers, and their blends [27]. Stabilized fertilizers can inhibit some stages of N transformation in the soil through additives such as urease or nitrification inhibitors (e.g., N-(n-butyl) thiophosphoric triamide—NBPT) [28,29]. Some chemical compounds, such as boric acid, and metallic ions, such as copper (Cu), may also function as urease inhibitors when used in adequate concentrations [28,30].

The slow-release or chemically modified fertilizers are products of condensation of urea with aldehydes (e.g., formaldehyde and acetaldehyde). Controlled-release fertilizers are those with coatings that control the release of nutrients by diffusion or by a physical barrier (e.g., sulfur, wax, and polymer) [24,31]. In addition to these technologies, combining enhanced efficiency fertilizers and conventional N sources gives rise to another category of fertilizers, what are known as blends. N fertilizer blends are produced from the physical mixture of different fertilizer technologies with conventional N sources (stabilized, slow-release, or controlled-release fertilizers). Combining these N sources has many advantages, including a reduction in production costs compared to separate application of slow- or controlled-release fertilizers, the optimization of dynamics between nutrient release and plant nutrient uptake, and a reduction in NH_3-N losses compared to conventional urea [32,33].

In addition to the different technologies available on the market, diverse N fertilization application strategies can be used, such as the mechanical incorporation of fertilizers into the soil. However, incorporation cannot be used in some cropping systems. In coffee fields, for example, mechanical incorporation of fertilizers may damage the root system, whose greatest activity occurs in the first 0.30 m of the soil [34]. Thus, urea dissolved in water, applied via a jet directed to the soil, can be a promising alternative, since urea would be automatically incorporated.

In this study, we tested the hypothesis that enhanced efficiency N fertilizers and other fertilizers, such as ammonium nitrate and sulfate and prilled urea diluted in water, are options more suitable than conventional urea to reduce NH_3-N losses in coffee production systems. We chose the main technologies available on the market to perform this study. Thus, the objective of this study was to quantify NH_3-N losses by volatilization from conventional, stabilized, slow-release, and controlled-release N fertilizers, as well as from a fertilizer blend, that were applied for two crop seasons on a coffee growing area in the production stage.

2. Results

2.1. Weather Conditions

The accumulated precipitation in the fertilization period of the 1st year was: 337 mm, 289 mm, and 69 mm at the 1st, 2nd, and 3rd split fertilizations, respectively, totaling 694 mm. In the first seven days after each fertilizer application, these rainfall values corresponded to 78 mm (23%), 171 mm (59%), and 19 mm (27%) (Figure S1). In the 2nd year, the rainfall accumulated during the fertilization period was: 153 mm, 124 mm, and 178 mm at the 1st, 2nd, and 3rd split fertilizations, respectively, totaling 455 mm. In the first seven days after each fertilizer application, these rainfall values corresponded to 102 mm (67%), 64 mm (52%), and 40 mm (22%) (Figure S3). The mean annual rainfall over the two years of assessment was 1243.3 mm.

The relative air humidity was higher than the critical relative humidity of urea (75%) for most of the period after fertilization in the two crop seasons. The mean temperatures in the same period were 21.2 and 22.6 °C in the first and second crop seasons, respectively. The minimum temperatures were 19 and 18 °C, and the maximum temperatures were 28 and 25 °C. Between the years 2015 and 2017, January had the highest mean temperature (30 °C), and June had the lowest mean temperature of (14 °C). The potential evapotranspiration (ETP) was around 899 to 873 mm per year [35].

2.2. Daily and Accumulated N-NH_3 Losses

In this study, the results were divided into two topics for a better understanding of the treatments and for a fair comparison among the N-sources. The first topic includes the results of fertilizers applied in three split applications, and the second topic describes fertilizers applied in a single application.

2.2.1. Fertilizers Applied in Three Split Applications

The daily and accumulated losses of N-NH_3 of the three fertilizer applications in each crop year were influenced ($p \leq 0.05$) by the technologies for N-fertilizers. Except for urea+adhesive+$CaCO_3$, all the technologies for N-fertilizers, ammonium nitrate, ammonium sulfate and stabilized fertilizers reduced N-NH_3 losses compared to prilled urea. For the 2015/2016 crop season, the maximum losses or peaks of daily NH_3 volatilization for prilled urea occurred 1.3 days after application (~5.8 kg N ha^{-1}). For ammonium nitrate and ammonium sulfate, the maximum loss occurred 6.3 and 4.5 days after application, with values of 0.03 and 0.01 kg N ha^{-1}, respectively. For fertilizers stabilized with Cu + B and NBPT, the maximum loss occurred at 4.9 and 2.6 days after application, with 1.0 and 2.5 kg N ha^{-1}, respectively. Lastly, for urea dissolved in water and urea+adhesive+$CaCO_3$, the maximum loss occurred at 1 and 1.15 days after application, with 1.5 and 1 kg N ha^{-1} (Table 1). In the 2015/2016 crop season, the mean accumulated losses in the first seven days were 9.2, 6.7, and 13% of the applied N for the first, second, and third split applications, respectively (Figure S1). In the 2016/2017 crop season, the maximum loss for prilled urea occurred two days after application, with a value of 7.4 kg N ha^{-1}. For ammonium nitrate and sulfate, the maximum loss was at 13 and 5 days after application, with values similar to those of the first crop season (lower than 0.2 kg N ha^{-1}). Urea + Cu + B and Urea + NBPT showed maximum losses at 3.7 and 4.3 days, with values of 4.0 and 3.2 kg N ha^{-1}, respectively. Urea dissolved in water and urea + adhesive + $CaCO_3$ had maximum losses at 1.5 and 1.9 days after N fertilization, with values of 1.6 and 10.3 kg N ha^{-1} (Table 2). In the 2016/2017 crop season, the mean general accumulated losses in the first seven days were 16, 8.5, and 11.7% for the first, second, and third split applications, respectively (Figure S3).

Table 1. Regression parameters adjusted for the accumulated and maximum daily losses of $N\text{-}NH_3$ from conventional and stabilized N fertilizers in the 2015/2016 crop season.

Treatment	Split Fertilization	Parameters				MDL (kg)
		α	b	k	R^2	
		Maximum NH_3 Loss	Day of the Maximum Loss			
Prilled urea	1	23.65	1.31	1.45	0.97	8.573
	2	13.97	0.77	1.24	0.99	4.331
	3	30.78	1.70	0.59	0.99	4.540
Ammonium nitrate	1	0.26	6.70	0.27	0.94	0.018
	2	0.21	4.89	0.64	0.95	0.034
	3	0.27	7.21	0.40	0.94	0.027
Ammonium sulfate	1	0.57	0.12	0.22	0.97	0.031
	2	0.06	6.79	0.17	0.95	0.003
	3	0.31	6.54	0.08	0.98	0.006
Urea + NBPT	1	9.97	2.72	0.40	0.98	0.997
	2	6.33	2.20	1.52	0.98	2.405
	3	22.32	2.78	0.73	0.98	4.073
Urea dissolved in water	1	7.19	0.89	1.80	0.96	3.236
	2	2.45	−7.19	0.23	0.94	0.141
	3	3.80	0.70	1.32	0.97	1.254
Urea + adhesive + $CaCO_3$	1	22.67	1.23	2.49	0.96	14.112
	2	21.89	1.21	2.35	0.99	12.860
	3	30.41	1.05	0.56	0.98	4.257
Urea + Cu + B	1	3.46	5.32	0.40	0.98	0.346
	2	2.04	1.29	2.22	0.98	1.132
	3	16.34	7.98	0.39	0.99	1.593

α: Asymptotic value (percentage of estimated maximum accumulated loss); b: Day when the maximum ammonia loss occurs; k: relative index; MDL (maximum daily loss of ammonia) and NBPT: N-(n butyl) thiophosphoric triamide.

Table 2. Regression parameters adjusted for the accumulated and maximum daily losses of $N\text{-}NH_3$ from conventional and stabilized N fertilizers in the 2016/2017 crop season.

Treatment	Split Fertilization	Parameters				MDL
		α	b	k	R^2	
		Maximum NH_3 Loss	Day of the Maximum Loss			(kg)
Prilled urea	1	32.03	1.72	1.32	0.99	10.570
	2	16.76	2.40	0.85	0.96	3.562
	3	22.28	1.97	1.46	0.98	8.132
Ammonium nitrate	1	0.48	9.68	0.22	0.94	0.026
	2	0.51	24.80	0.07	0.81	0.009
	3	1.72	4.90	0.43	0.93	0.185
Ammonium sulfate	1	0.05	2.32	1.10	0.91	0.014
	2	0.36	4.21	0.52	0.91	0.047
	3	1.65	8.37	0.33	0.97	0.136
Urea + NBPT	1	18.67	3.01	1.06	0.99	4.948
	2	10.41	5.25	0.79	0.97	2.056
	3	17.32	4.73	0.59	0.98	2.555
Urea dissolved in water	1	5.66	1.69	0.90	0.99	1.274
	2	4.77	1.45	2.74	0.94	3.267
	3	1.33	1.43	1.24	0.93	0.412

Table 2. *Cont.*

Treatment	Split Fertilization	Parameters				MDL
		α	b	k	R^2	
		Maximum NH_3 Loss	Day of the Maximum Loss			(kg)
Urea + adhesive + $CaCO_3$	1	36.22	1.70	1.81	0.99	16.390
	2	18.56	1.78	1.16	0.93	5.382
	3	34.12	2.10	1.09	0.98	9.298
Urea + Cu + B	1	20.27	3.54	1.31	0.98	6.638
	2	15.29	2.84	0.73	0.95	2.790
	3	21.58	4.79	0.54	0.98	2.913

α: Asymptotic value (percentage of estimated maximum accumulated loss); b: Day when the maximum ammonia loss occurs; k: relative index; MDL (maximum daily loss of ammonia) and NBPT: N-(n butyl) thiophosphoric triamide.

Regarding the accumulated N-NH_3 losses in the 2015/2016 crop season, the mean value of losses was 10.6% of the applied N (average of the three split applications) (Table 3, Figure S5). For the treatments, the mean losses decreased as follows: Urea + adhesive + $CaCO_3$ (25.5% of applied N) = prilled urea (23.2%) > urea + NBPT (13%) > urea + Cu + B (7.4%) > urea dissolved in water (4.5%) > ammonium sulfate (0.3%) = ammonium nitrate (0.2%). For the 2016/2017 crop season, the mean value was 13.7% (average of the three split applications). As for the treatments, the mean losses decreased in the following order: urea+adhesive+$CaCO_3$ (30.3% of applied N) > prilled urea (24.2%) > urea + Cu + B (19.7%) > urea+NBPT (16%) > urea dissolved in water (4.5%) > ammonium sulfate (0.9%) = ammonium nitrate (0.8%) (Table 3, Figure S6).

Table 3. Mean accumulated losses of ammonia (% of applied N), for conventional and stabilized N fertilizers, in three fertilizations in the coffee plantation, during the 2015/2016 and 2016/2017 crop seasons.

Treatment	Ammonia Loss (%)								Mean ** (%)	PCRDU ** (%)
	Season 2015/2016				Season 2016/2017					
	1st	2nd	3rd	Mean	1st	2nd	3rd	Mean		
Prilled urea	24.2a	14.0b	31.6a	23.2a	32.3a	17.3a	23.1b	24.2b	23.7b	-
Urea dissolved in water	7.3b	2.4c	4.0d	4.5d	5.6c	5.1b	1.5c	4.0e	4.2d	82.3
Ammonium sulfate	0.6c	0.1c	0.3d	0.3e	0.6c	0.4b	1.9c	0.9e	0.6e	97.5
Ammonium nitrate	0.3c	0.2c	0.3d	0.2e	0.5c	0.3b	1.8c	0.8e	0.5e	97.9
Urea + Cu + B	3.5c	2.0c	16.7c	7.4c	20.7b	15.8a	22.8b	19.7c	13.5c	43
Urea + adhesive + $CaCO_3$	23.7a	22.0a	31.0a	25.5a	36.7a	19.3a	35.1a	30.3a	27.9a	−17.7 ***
Urea + NBPT	9.9b	6.4c	22.7b	13.0b	18.8b	11.3a	18.0b	16.0d	14.5c	38.8
Mean	9.9	6.7	15.2	10.6	16.4	9.9	14.9	13.7	12.1	56.9
Coefficient of Variation	18	17.9	16.2	11.7	15.4	27.9	11.2	18.2	8.5	-

NBPT: N-(n butyl) thiophosphoric triamide. Note: In each crop season, 300 kg N ha^{-1} per year were split into three equal applications for conventional and stabilized N fertilizers, totaling 600 kg N ha^{-1} for both crop seasons. Means followed by the same lowercase letter in the column do not differ by the Scott-Knott test ($p \leq 0.05$). Mean of the six fertilization sources performed between November and February during both seasons ** (PCRDU) Percentage change decrease compared to Prilled Urea. *** Negative value indicates increased volatilization compared to prilled urea.

2.2.2. Fertilizers Applied in a Single Application

In this section, the results of slow-release and controlled-release fertilizers and a blend will be presented. Here, fertilizers were applied in a single application, as they have the mechanism of gradual release of N to the soil. The results showed significant differences in N-NH_3 losses by volatilization for both seasons. In the 2015/2016 crop season, the maximum loss occurred 35 days after the application for urea+elastic resin, with a mean value of 0.4 kg N ha^{-1}. For the Blend N-fertilizer, at 24.7 days (0.19 kg N ha^{-1}); urea-formaldehyde, at 7 days (0.08 kg N ha^{-1}); urea+polyurethane, at 28.2 days (0.37 kg N ha^{-1}). Regarding the 2016/2017 crop season, these N-sources behave similarly, with low values on the day of maximum loss. The urea+elastic resin treatment showed maximum loss at 40 days (~0.2 kg N ha^{-1}); Blend N-fertilizer, at 9 days (~0.5 kg N ha^{-1}); urea-formaldehyde, at 9 days (~0.04 kg N ha^{-1}); and urea+polyurethane, at 31 days (0.22 kg N ha^{-1}) (Table 4).

Table 4. Regression parameters adjusted for the accumulated and maximum daily losses of N-NH_3 from slow-release and controlled-release N fertilizers in the 2015/2016 and 2016/2017 crop seasons.

Treatment	Crop Season	Parameters				MDL
		α	b	k	R^2	
		Maximum NH_3 Loss	Day of the Maximum Loss			(kg)
Urea + elastic resin	2015/2016	5.67	35.94	0.10	0.99	0.425
Blend N-fertilizer		4.29	24.71	0.06	0.94	0.193
Urea-formaldehyde		0.53	6.99	0.20	0.82	0.080
Urea + polyurethane		6.20	28.25	0.08	0.98	0.372
Urea + elastic resin	2016/2017	5.75	40.56	0.05	0.99	0.216
Blend N-fertilizer		6.07	9.23	0.12	0.95	0.546
Urea-formaldehyde		0.42	8.88	0.14	0.90	0.044
Urea + polyurethane		3.80	31.10	0.08	0.99	0.228

α: Asymptotic value (percentage of estimated maximum accumulated loss); b: Day when the maximum ammonia loss occurs; k: relative index; MDL (maximum daily loss of ammonia) and NBPT: N-(n butyl) thiophosphoric triamide.

The losses accumulated by these sources in the 2015/2016 crop season were higher for the treatments: urea + polyurethane (6.4% of applied N) > urea + plastic resin (5.7%) = Blend N-fertilizer (4.6%) > urea-formaldehyde (0.6%) (Figure S2). In the 2016/2017 crop season, losses were higher for the Blend N-fertilizer (6.5% of applied N) = urea + plastic resin (5.9%) > urea + polyurethane (4%) > urea-formaldehyde (0.5%) (Table 5, Figure S4).

Table 5. Mean accumulated losses of ammonia (% of applied N), for slow-release and controlled-release N fertilizers, in one single application in the coffee plantation, during the 2015/2016 and 2016/2017 crop seasons.

Treatment	Crop Season						Mean of Two Crop Seasons **
	Ammonia Loss (%)						
	2015/2016			2016/2017			
	1st	2nd	3rd	1st	2nd	3rd	(%)
Blend N-fertilizer		4.59a			6.46a		5.53a
Urea + elastic resin		5.71a			5.91a		5.81a
Urea-formaldehyde		0.58b			0.48c		0.53b
Urea + polyurethane		6.40a			4.02b		5.21a
Mean		4.32			4.22		4.22
Coefficient of Variation		0.43			0.47		0.47
Precipitation (mm)		694 *			455 *		574 ***

Note: In each crop season, 300 kg N ha^{-1} per year were applied into one single application for slow and controlled-release N fertilizers, totaling 600 kg N ha^{-1} for both crop seasons. Means followed by the same lowercase letter in the column do not differ by the Scott-Knott test ($p \leq 0.05$). * Sum of precipitation during the evaluation periods, which were 208 and 235 days in the 2015/2016 and 2016/2017 crop seasons, respectively. ** Mean of the six fertilizations performed between November and February of each crop season/year. *** Mean of precipitation during the evaluation periods, which were 208 and 235 days in the 2015/2016 and 2016/2017 crop seasons.

2.3. Summarizing Results of Ammonia Losses from N-Technologies

Considering the way that the study was designed and conducted, it is not possible to compare the results of all technologies. However, a sequence of loss values presented by the sources can be established, considering the average of the two years of study. Thus, the decreasing order for the split treatments would be as follows: urea + adhesive + $CaCO_3$ (27.9% of applied N = 84 kg N) > prilled urea (23.7% = 71 kg N) > urea + NBPT (14.5% = 43 kg N) = urea + Cu + B (13.5% = 40 kg N) > urea dissolved in water (4.2% = 12.6 kg N) > ammonium sulfate (0.6% = 1.8 kg N) = ammonium nitrate (0.5% = 1.5 kg of N). The decreasing order for the sources applied at a single time were: urea + elastic resin (5.8% = 17.4 kg N) = Blend N-fertilizer (5.5% = 16.6 kg N) = urea + polyurethane (5.2% = 15.6 kg N) > urea-formaldehyde (0.5% = 1.59 kg).

3. Discussion

In this study, the weather conditions greatly influenced N-NH_3 losses by volatilization, particularly rainfall and temperature. In both coffee crop seasons, most N-NH_3 losses occurred in the first seven days for the N-fertilizers applied in three split applications. The rainfall in these first days was essential for incorporating fertilizers into the soil and reducing N-NH_3 emissions. Such a pattern was evidenced in both seasons.

In 2015/2016, the accumulated rainfall in the first seven days (19 mm) of the third split application was the lowest. Such low rainfall led to an increase of 40 and 90% in N-NH_3 losses compared to the first and second split applications, respectively. The same pattern was not observed for the 2016/2017 season. However, an important issue must be considered: in the first and second N-fertilization, the mean NH_3 losses in the first seven days were 16% and 8.5%, respectively. Such lower NH_3 loss of 8.5% can be due to the absence of precipitation in the first two days of the first split application, which favored the permanence of the NH_4^+ from the N-fertilizers for a longer time on the soil surface. Regarding the pattern observed in the third split application, the losses were significant until the 13th day after fertilizer application, which is due to the lack of rainfall in the first days. These results show that rainfall before or after fertilization affects N losses by volatilization, particularly in the first seven days after the fertilizer application. Considering the initial seven days as the most critical phase to lose ammonia after applying prilled urea, the use of technologies associated with urea is critical to reducing N losses by volatilization [36,37]. In this context, NH_3 losses depend on the volume and intensity of the rainfall [38].

This relationship between precipitation and N-fertilizer incorporation into the soil becomes even more complex in coffee plantations, as the architecture of the coffee plant restricts the direct incidence of rainfall, thus limiting the incorporation of the N-fertilizer applied in the canopy projection. Plant residues on the soil surface also function as a barrier to fertilizer incorporation (Figure S13).

Regarding the efficiency of urea + NBPT in reducing N-NH_3 losses by volatilization, it is possible that the NBPT inhibited the urease activity, which is responsible for urea hydrolysis [12,30]. The efficiency of the NBPT was evidenced by the delay of 1.3 days in daily ammonia volatilization peaks, the reduction in MDL by 63%, and the 38.8% reduction of the accumulated loss compared to prilled urea. Therefore, the NBPT effectively delayed the beginning of N-NH_3 losses and reduced the accumulated losses compared to prilled urea. This delay possibly increased the chances of N-fertilizer incorporation by rainfall, which can reduce the losses of N by volatilization [39].

NBPT is currently the most used urease inhibitor worldwide [39]. A meta-analysis study reported that NBPT can reduce N-NH_3 losses by 52% on average, compared to the ammonia losses of prilled urea [6]. Urease inhibitors are highly efficient in reducing N-NH_3 losses by volatilization, but some aspects must be considered when NBPT is added to urea. These aspects include: its degradation under increased soil temperature [40], acidic soil pH [41], time and temperature of storage [12], and contact with phosphate fertilizers, which contain free acidity [42].

Urea + Cu + B reduced the N-NH_3 losses by 68% and 18% compared to prilled urea in the 2015/2016 and 2016/2017 crop seasons. This efficiency in reducing losses is due to the potential for urease inhibition using Cu and B. The urease inhibition mechanism is due to the reaction of copper with the sulfhydryl groups of the urease enzyme, forming insoluble sulfites and inactivating the enzymatic action of urease [43,44]. Boric acid (H_3BO_3) can also inhibit urease activity but through a different inhibition mechanism. In this case, H_3BO_3 has a very similar structure to urea and functions as an analog substrate for ureases. Thus, it replaces almost perfectly the water molecules bound to Ni at the center of the reaction [45,46]. Urea treated with Cu and B is already commercialized in Brazil, and for this study, it was bought from the regional fertilizer market. Although Cu and B are potential urease inhibitors, the low concentrations found in some commercial products may not be enough to inhibit the enzyme [30]. Thus, proper concentrations must be evaluated in varying crops and cropping systems. Some issues related to the treatment process in the fertilizer industry still complicate the increase in the amounts of Cu and B added to urea, especially with the use of H_3BO_3, which has a low concentration of B (17%).

Ammonia losses in the treatment urea + adhesive + $CaCO_3$ were 18% higher than prilled urea in two coffee crop seasons. In this treatment, calcium carbonate was used as an alternative to elemental sulfur to create a physical barrier around the urea granule. In the present study, this technology was inefficient due to its limited effect as a physical barrier for the urea granule. $CaCO_3$ increased the porosity and the contact with water enhanced its dissolution. This characteristic was evidenced when the day of the maximum NH_3 loss was anticipated as well as the increase in the maximum NH_3 daily loss in relation to the prilled urea. In addition, $CaCO_3$ increases the alkalization that occurs around the urea granule hindering the pH buffering capacity of the region where the urea is hydrolyzed. Such a physical barrier with $CaCO_3$ in urea increases the N-NH_3 losses. Furthermore, this concept was also verified by the NH_3 accumulated losses from the da urea + Adhesive + $CaCO_3$, which was higher than the prilled urea. Thus, we concluded that the alkaline ($CaCO_3$) coating urea was inefficient to reduce the ammonia losses by volatilization.

There are two N-fertilizers widely used in Brazilian coffee plantations, namely ammonium nitrate and ammonium sulfate. In this study, the reduction of N-NH_3 losses for these two sources was higher than 97% in both crop seasons. The irrelevant NH_3 losses from these N-sources are related to their acidic-to-neutral reaction in soil, mainly at pH < 7 [47]. Another positive aspect is that these fertilizers do not depend on weather conditions at the moment of their application. Thus, both ammonium nitrate and sulfate can be smart options for N-fertilization in coffee crop systems.

Altogether, the application of urea dissolved in water by drench draws attention to the technologies used to mitigate prilled urea losses by volatilization. In the present scientific study, this treatment showed good efficiency in reducing N-NH_3 losses by volatilization. The days of maximum loss occur very similarly to prilled urea applied on the soil surface. However, the losses in these days of maximum loss are, on average, 3.8 and 4.5 times lower than prilled urea for the 2015/2016 and 2016/2017 crop seasons, respectively. Moreover, the accumulated losses of urea dissolved in water were five and six times lower than those observed for prilled urea in the 2015/2016 and 2016/2017 seasons, respectively. Such reduced losses are due to the dissolution of urea in water, which percolates to subsurface layers in the soil carrying the urea molecules, thus reducing N-NH_3 losses by volatilization. For this treatment, no additive was added to the conventional urea. However, urease or nitrification inhibitors can also be added to the urea solution [48], thus improving urea use efficiency, that is, the technologies available in the market can be associated with strategies that can further increase the N use efficiency.

Considering the management of coffee plantations in Brazil, the application of urea solution can be performed along with systemic insecticides. Such products are applied directly in the ground, under the projection of the coffee tree canopy. However, it is important to evaluate the compatibility between the products to be applied, as well as the spray volume used and its relationship with the urea concentration in the solution, related to the solubility product constant [16]. Another strategy would be to add micronutrients to the urea solution in order to standardize the distribution. In addition, increasing the concentration of B and Cu in the urea solution could inhibit urease activity and help mitigate ammonia losses.

In this study, it was not possible to compare the conventional, stabilized, slow-, and controlled-release fertilizers. However, the latter treatments showed interesting patterns when applied to coffee cropping systems. The basis of slow-release, controlled-release, and Blend N-fertilizers is urea, but the associated technologies lead to contrasting responses in N-NH_3 losses. The average accumulated loss by those sources is lower than 6% of the applied N when averaging the two crop seasons.

This pattern observed for controlled-release fertilizers (urea + elastic resin, urea + polyurethane) is explained by the way N is released into the soil. The release of N in controlled-release fertilizers occurs by the diffusion of urea from inside the granule through the coating into the soil solution. This process starts with increasing steam pressure and water intake into the granule. Then, osmotic pressure inside the capsule increases and creates a diffusion gradient from the fertilizer to the soil solution [49]. The gradual release reduces the excess of N-mineral available in the soil solution, which is susceptible to volatilization, denitrification, and leaching. Controlled-release urea improves the synchronism between the N release from fertilizer granules and its absorption by the plants, thus reducing N losses and improving nitrogen use efficiency in coffee crop environments [2,50].

The chemical reactions in the Urea-Formaldehyde production process reduce the nitrogen solubility in water compared to conventional sources of N, owing to the formation of long and short polymerization chains. This reduced solubility has varying effects on the rates of N release over time. The methylene urea chains formed in the Urea-Formaldehyde production depend on the activity of microorganisms in a process similar to N mineralization in the soil. Such a process prevents all the N from being readily released into the soil and is thus subject to the transformations needed to produce NH_3 [51–55]. From an agronomic perspective, the lower N-NH_3 losses are due to the reduction of excessive mineral N in the soil solution, which is susceptible to N-losses. The same pattern was also observed in the controlled-release urea. In the present study, the release time of controlled-release or slow-release fertilizers such as urea-formaldehyde was not verified. However, these enhanced efficiency fertilizers should be further investigated regarding their potential for proper N supply for coffee crop systems.

The Blend N-fertilizer, a blend of urea stabilized with NBPT and urea coated with elemental sulfur and polymer, was also efficient in reducing N losses, which did not exceed 7% in both coffee crop seasons. In this blend, part of the urea is in the soluble form and is protected by the NBPT as a urease inhibitor. The Blend N-fertilizer improves the N provision to the coffee plants over time as it combines the fast release of the soluble urea mixed with NBPT and the controlled-release urea to provide nitrogen for a longer time. The N-NH_3 losses were similar to the 100% coated treatments compared to blend N-fertilizers, thus demonstrating the efficiency of this technology to supply N to the coffee plant.

Highlights of Economic View of N-Fertilizers Technologies

From an economic perspective, here we present a short overview related to the prices of N-fertilizer technologies assessed in this scientific paper. Prilled urea has the lowest cost in the market, considering its increased N concentration and disregarding the high N-NH_3 losses. In sequence, are fertilizers stabilized with NBPT, Cu, and B, which have similar market values, followed by urea + adhesive + $CaCO_3$. The prices of Blend-N-fertilizer reduce as the proportion of the stabilized and conventional ureas increase in the physical mixture and their prices are higher than conventional and stabilized N-fertilizers. In addition, Blend-N-fertilizers have a lower cost compared to 100% of controlled-release urea.

In this context, urea + polyurethane and urea + plastic resin, which are controlled-release or added-value fertilizers, have similar market prices. In addition, the prices of controlled-release fertilizers may vary according to the material used in the coating and the thickness of the coating. Finally, urea-formaldehyde as well as controlled-release fertilizers require investments in industrial processing such as infrastructure with specific conditions to produce these added-value N-fertilizers. In some situations, in Brazil, urea-formaldehyde has been used as a blended form with conventional urea and/or ammonium sulfate reducing its price compared to pure urea-formaldehyde. In general, pure urea-formaldehyde has similar prices to pure controlled-release fertilizers.

From an agronomic/economic point of view, the decision on which N sources would be interesting for application in coffee plantations must consider the costs of the fertilizer application. Fertilization with conventional and stabilized fertilizers must be split into three or more applications. Slow- and controlled-release fertilizers can be applied in a single operation reducing the costs (labor, fuels, machine maintenance, and depreciation), time of mechanized operation on the farm, and soil compaction due to the reduction of N splits compared to conventional and stabilized fertilizers. On the other hand, the Blend N-fertilizer technology is more expensive than conventional and stabilized fertilizers, but almost always has a lower value compared to slow- and controlled-release fertilizers. Besides, Blend N-fertilizers provide better synchronism between the N release and its absorption by the plants.

4. Materials and Methods

4.1. Characterization of the Experimental Area

The experiment was conducted in coffee plantations under field conditions for two crop seasons, 2015/2016 and 2016/2017, in Lavras, Minas Gerais (MG), Brazil (Figure 1). Lavras (910 m a.s.l., 21°14′06″ S 45°00′00″ W) is located in a traditional region of coffee production in Brazil, within the Campos das Vertentes geographical indication. According to Köppen's classification, the climate is Cwa, mesothermal with mild summers and dry winters. The mean annual precipitation is approximately 1472 mm, the mean annual temperature is 19.4 °C [56].

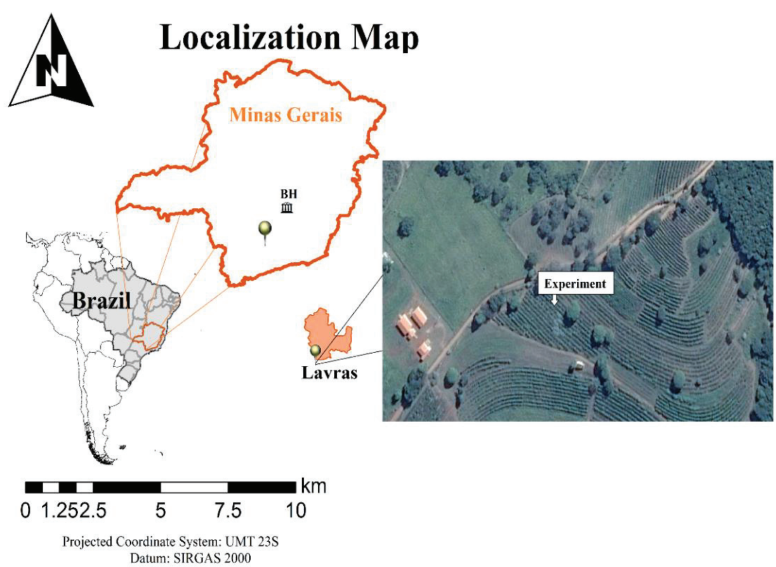

Figure 1. Location map of the experimental areas in Lavras, Minas Gerais, Brazil.

The coffee plantation in the production phase was planted with the "Catuaí Vermelho" cultivar, line 144. At the beginning of the experiment, the plantation was six years old. The spacing used in the planting was 3.7 m between rows and 0.7 m between plants, totaling 3861 plants ha^{-1}.

The soil was classified as "Latossolo Vermelho Amarelo Distroférrico (LVdf)" according to the Brazilian System of Soil Classification [57], or Haplustox [58]. Before installing the experiment, soil samples were collected at the 0–0.2 m depth for soil texture [59] and soil chemical analyses. (Table 6) lists the result of the soil chemical analysis and texture.

Table 6. Chemical characterization and soil texture of the experimental area at the 0–20 cm depth, before the application of the treatments.

pH	K	P	Cu	B	Ca^{2+}	Mg^{2+}	Al^{3+}	CEC	OM	BS	Sand	Silt	Clay
		mg dm^{-3}				$cmol_c$ dm^{-3}					%		
4.6	92	16	1.5	0.3	1.7	0.4	0.7	11.4	2.4	30	18	24	58

pH in water (1:2.5); P, K, and Cu extracted by Mehlich-1; B extracted by hot water; Ca^{2+}, Mg^{2+}, and Al^{3+} extracted by 1 M KCl; CEC = Cation Exchange Capacity at pH 7.0; OM = soil organic matter; BS = base saturation; Sand, silt, and clay = particle-size fractions.

4.2. Experimental Design

In this study, different sources of N-fertilizers were used, which were applied in a single application or split into three applications. Thus, two different group experiments were carried out in the same area, but the management practices (other than fertilization) remained similar. The experiments were designed as follows: Group 1) seven treatments, consisting of conventional and stabilized fertilizers and urea dissolved in water (management strategy) the experimental design in the field was randomized blocks with three repetitions, totaling 21 plots; and Group 2) four treatments, consisting of slow-, controlled-, and blend fertilizers, the experimental design in the field was randomized blocks with three repetitions, totaling 12 plots. For conventional and stabilized fertilizers, a dose of 300 kg ha^{-1} was divided into three applications. For the other treatments (Group 2) a dose of 300 kg ha^{-1} was applied in a single application. The treatments will be described in detail in the next topic. Each experimental unit consisted of 14 coffee plants. The ten central plants comprised a useful area for data collection.

4.3. Characterization of the Fertilizers

The fertilizers used in this study were chosen based on technologies used in Brazilian coffee production systems. They were divided into four groups and characterized according to the type of technology used. We photographed all fertilizers with a Canon camera, SL3 DSLR model, and an Olympus microscope, SZ60 Japan model. Fertilizers classified as controlled-release were characterized by scanning electron microscopy (SEM) and energy dispersion X-ray spectroscopy (EDS).

The first group included the conventional fertilizers: (1) prilled urea (45% N), (2) Ammonium nitrate (31% N), and (3) Ammonium sulfate (21% N and 24% S-SO_4^{2-}). Another treatment containing prilled urea (45% N) diluted in water at a concentration of 50 g L^{-1} was also added, aiming to reduce N-NH_3 losses and to completely dissolve urea: (4) urea dissolved in water.

Another group amongst the technologies used in this study was the stabilized fertilizers, which have additives that can inhibit or delay some process of N transformation in the soil: (5) urea treated with Cu and B (44% N; 0.4% B as boric acid and 0.15% Cu as copper sulfate) and (6) urea treated with NBPT (45% N). This group of fertilizers consists of urease inhibitors (NBPT, NPPT, Cu, and B). The functioning of Cu and B as urease inhibitors depends on the concentrations added to the fertilizer. Besides being micronutrients, Cu and B have competitive and non-competitive urease inhibition capacities, respectively [60].

The group of controlled-release fertilizers was also included in this study: (7) urea coated with elastic resin (44% N and 43.8 μm of average coating thickness), (Figure S8) (8) urea coated with polyurethane (40% N and 56.4 μm of average coating thickness), (Figure S9).

The group of chemically modified or slow-release fertilizers was represented in this study by (9) urea-formaldehyde (26% N). This product results from the reaction of formaldehyde molecules (H_2CO) with urea $(NH_2)_2CO$ under controlled temperature and pressure. This reaction forms chains of C and N with different sizes and degrees of polymerization.

A treatment for physical protection of the urea granules was included in this study: (10) urea + adhesive + $CaCO_3$. This treatment included a compound that agglutinates calcium carbonate ($CaCO_3$), creating a physical barrier of adhesive and $CaCO_3$. This barrier temporarily prevents contact between the soluble conventional urea and soil moisture.

Lastly, a fertilizer based on the physical mixture of technologies (blend) was added to the present study, constituting the treatment called: (11) Blend N-Fertilizer (39% N, 9% S^0) (Figure S7). In this case, the release of N to the system occurs in different stages, as this blend is a mixture of conventional urea with a controlled release fertilizer (urea coated with elemental sulfur (S^0) + polymer), measuring 67.5 μm of coating thickness and a stabilized fertilizer (urea treated with NBPT, most of the times). Therefore, the blend aims at the synchronization of the release of N by the fertilizer and its absorption by the plant, which reduces N excess in the system and N-NH_3 losses by volatilization.

For the other treatments, the granulometry of the fertilizers varied from 1 to 4 mm, as officially specified by the Brazilian legislation for granulated fertilizers. Further physical characteristics of the treatments can be found in figures (Figures S7–S12).

The treatments used in this study were applied at the 300 kg N ha^{-1} dose per year. The application of the treatments prilled urea, ammonium nitrate, ammonium sulfate, stabilized (urea + NBPT and urea + Cu + B) and urea + adhesive + $CaCO_3$ were split into three doses of 100 kg N ha^{-1} into the two crop seasons of the experiment. Urea dissolved in water was applied via drench at a dose of 1.6 L m^{-1}, totaling 16 L per plot, following the same criteria described for the split application. The slow-release, controlled-release, and blend fertilizers were applied at a single dose of 300 kg N ha^{-1} per year. All fertilizers were applied as topdressing, superficially, and under the canopy projection of the coffee plants. The applications for the 2015/2016 season were conducted on 6 November 2015, 11 January, and 10 March 2016. The 2016/2017 season received the applications following the same interval. The treatments received the slow-release, controlled-release, and blend-N fertilizers on the same day as the first application of the conventional and stabilized fertilizers.

4.4. Complementary Management of Soil Fertility

Liming was performed 60 days before applying the N fertilizers in each treatment plot, aiming to increase soil base saturation to 60%. The dose of 2 t ha^{-1} of agricultural lime (PRNT 100%) was used in both crop seasons. Maintenance fertilization was performed with potassium chloride (KCl—60% K_2O) and simple superphosphate (SFS—20% P_2O_5) fertilizers, applied at doses of 300 kg K_2O ha^{-1} per year and 100 kg P_2O_5 ha^{-1} per year, respectively, under the canopy projection of the coffee plants [61].

The micronutrients were applied via leaf fertilization along with phytosanitary control. These procedures were performed both during the formation period of the coffee plantation and over the experimental period. A total of 5 kg ha^{-1} of a commercial product containing the following nutrients were applied: 6.0% zinc ($ZnSO_4$), 3.0% boron (H_3BO_4), 2.0% manganese ($MnSO_4$), 10.0% copper ($Cu(OH)_2$), 10.0% sulfur, 1.0% magnesium ($MgSO_4$) and 10.0% K_2O (KCl). The spray volume applied was 300 L ha^{-1}, totaling three applications per year in 45-day intervals between November and February each year.

4.5. Quantification of N-NH_3 Losses

The losses of N-NH_3 owing to the application of N fertilizers were quantified using the semi-open collector adapted by Lara Cabezas [62]. In the first year, three PVC bases (0.2 m height and 0.2 m diameter) were installed 30 days before the application of the fertilizers in each experimental plot, under the canopy projection of the coffee plants, and at a depth of 0.05 m into the soil (Figure 2). These PVC chambers were kept in the field during the two years of the experiment.

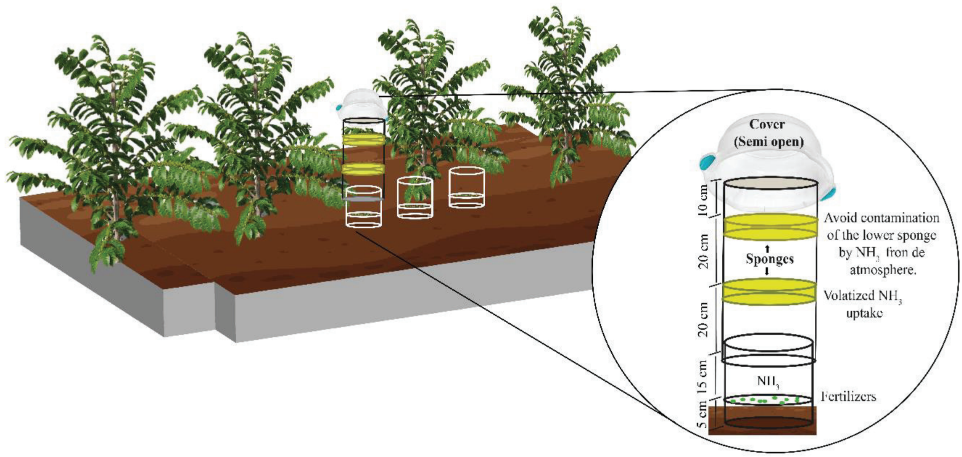

Figure 2. Illustration of the collectors used in the quantification of ammonia losses.

Collection chambers were made in PVC with a diameter similar to the bases. The chambers had lids that prevented water to enter, but allowed air circulation. They had 0.5 m height and specifications as described in (Figure 2). The amount of fertilizer corresponding to the dose applied per hectare was added within each base (0.2 linear meters). To calculate the dose of N, we considered the useful distance in linear meters of one hectare and the 3.7 m spacing between lines, totaling 2702.7 m. The dose of N was corrected for the equivalent base diameter (0.20 m). For the N-sources whose fertilization was split into three applications, 7.4 g of N was added to each base on the same day that the fertilization of the plots was performed. As for the treatments that received one single fertilizer application, 22.20 g of N was added to each base. The collection chamber was added to one of the bases, in all plots, immediately after applying fertilizers on the bases.

Two laminated foam discs with 0.02 g cm^{-3} density, 0.2 m thickness, and the same diameter as the PVC tubes were placed inside each semi-open collector. The foam discs were soaked with 80 mL of phosphoric acid (H_3PO_4; 60 mL L^{-1}) solution and glycerin (50 mL L^{-1}). The lower disc was placed inside the chamber at a height of 0.35 m from the soil, and the upper disc at 0.2 m from the lower one (Figure 2). The lower foam disk aimed to capture the ammonia released by the treatments, as the upper disk aimed to avoid contamination of the lower disk by N-NH_3 released from the rest of the fertilized line.

Foam disks were collected on the 1st, 2nd, 3rd, 4th, 5th, 7th, 9th, 12th, 15th, 19th, 24th, and 31st days after the application of fertilizers in the 2015/2016 crop season for conventional and stabilized fertilizers (Group 1). In 2016/2017, the collections were performed on the 1st, 2nd, 3rd, 4th, 5th, 7th, 9th, 12th, 14th, 17th, and 20th days, and until the 34th day after applying the treatments. The collections in the treatments with slow- and controlled-release fertilizers (Group 2) were performed on the same day as the conventional and stabilized fertilizers. However, they were extended until the 208th day of the first crop season and until the 235th of the second crop season.

After each sponge change, the chamber was rotated from one base to another to consider the influence of the spatial variability of ammonia emission. This rotation allows a greater influence on climatic variations, such as temperature and precipitation.

The solution in the sponges collected from the field was extracted by filtration in a Büchner funnel connected to a vacuum pump. The extraction was performed after ten sequential washes with 40 mL of deionized water each. The extracts were stored in a cold chamber for a maximum period of 5 days, and after that, they were analyzed. From the extract, 20 mL aliquots were taken to determine the N content by distillation by the Kjeldahl method [63]. The N content in the sample was calculated according to equation 1, adapted from Nogueira and Souza [64]: TN = $[(Va - Vb) \times F \times 0.1 \times 0.014 \times 100]/P1$, in which, TN: Total N concentration in the sample (%), Va: Volume of hydrochloric acid solution spent on the sample titration (mL), Vb: Volume of hydrochloric acid solution spent on the blank titration (mL), F: Correction factor for 0.01 M hydrochloric acid, P1: Sample mass (g).

The values obtained in the N content calculations referred to the area occupied by the base of the chambers installed in the field. These values were then extrapolated to the percentage of loss of N-NH_3 per hectare. The accumulated losses in the assessment were calculated by adding the losses from the 1st to the 2nd day, then adding this value to the losses of the 3rd day, and so on until the last day of collection.

4.6. Statistical Analysis

The treatments were submitted to a non-linear regression analysis using a logistic model (equation 2) to evaluate the ammonia loss by volatilization: $Yi = [\alpha/1 + e^{k}$ $(b - daai)] + Ei$, in which, Yi is the i-th observation of the accumulated loss of N-NH_3 in %, being i = 1, 2, ... , n; daai is the i-th day after the application of the treatment; α is the asymptotic value that can be interpreted as the estimation of the maximum accumulated loss of N-NH_3; *b* is the abscissa of the inflection point and indicates the day of the maximum loss by volatilization; k is the value that represents the precocity index, and the higher its value, the lower the time needed to reach the maximum loss by volatilization (α); Ei is the error associated to the i-th observation, which is assumed to be independent and equally distributed according to a zero average standard and constant variance, E ~ N (0, I σ2).

This model is already used to estimate plant growth but has recently been applied to estimate the N-NH_3 accumulated loss [6,65,66].

To estimate the maximum daily loss (day when the highest N-NH_3 loss occurred), that is, to determine the inflection point of the curve, the following equation was used: $MDL = k \times (\alpha/4)$, in which, k is a relative index used to obtain to a maximum daily loss of ammonia (MDL), and α is the asymptotic value that can be interpreted as the maximum amount of accumulated N-NH_3 loss. The "nlme" package was used in the modeling of the N-NH_3 losses data, using the R 3.3.1 software [67].

Normality and homoscedasticity of the data were verified by the Shapiro-Wilk and Bartlett tests, respectively. Then, an analysis of variance was performed to test the influence of the N sources on the N-NH_3 losses by volatilization. The significance of the differences was evaluated at $p < 0.05$. After validating the statistical model, the mean values were grouped by the Scott-Knott algorithm using the R 3.3.1 software [67].

5. Conclusions

Nitrogen fertilizers such as conventional urea can be used to improve nutrient use efficiency in coffee production environments by using technologies such as urease inhibitors and polymer coatings. Altogether, conventional urea had ammonia losses equal to 24% of N applied to promote lower N-use efficiency during two coffee seasons. Calcium carbonate as a physical coating around the urea granules performed poorly compared to all the other N-fertilizer technologies with ammonia volatilization losses 18% greater than conventional urea. Urea dissolved in water is an interesting N-fertilization management strategy for coffee farmers as the ammonia losses were only 4.2% of the applied N. Urea stabilized with N-(n-butyl) thiophosphoric triamide (NBPT) is a useful industrial innovative technology to mitigate ammonia losses because urease inhibitor as additive reduces ammonia losses by 39%. Slow- and controlled-release urea and Blend N-fertilizer are interesting added-value N-fertilizers to improve coffee crop nutrition over time because they can be applied in a single mechanized operation with ammonia losses lower than 7% of the applied N. Conventional N-fertilizers such as ammonium nitrate and ammonium sulfate showed negligible ammonia losses demonstrating its potential as interesting choices in comparison with conventional urea to mitigate ammonia emissions. Also, they can be applied regardless of soil humidity and climate conditions. In summary, in this scientific paper, we presented some highlights of cutting-edge technologies as a plan for the efficient use of N-fertilizers in coffee crop production environments. However, our research group is engaged in similar studies in the coffee crop, where not only aspects related to ammonia loss are being evaluated, but also emission of the other GHG, soil enzyme activity, and aspects related to plant nutrition, thus allowing better understanding of the N cycle for the coffee plant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11233323/s1, Figure S1: Daily N-NH_3 losses by volatilization in the first, second, and third split application (a, b, and c) of conventional and stabilized N fertilizers. Rainfall, average temperature, and relative air humidity after splitting the N fertilization in the 2015/2016 crop season (d); Figure S2: Daily (a) and accumulated (b) N-NH_3 losses by volatilization in slow- and controlled-release N fertilizers. Rainfall, average temperature, and relative air humidity after splitting the N fertilization in the 2015/2016 crop season (c); Figure S3: Daily N-NH_3 losses by volatilization in the first, second, and third split application (a, b, and c) of conventional and stabilized N fertilizers. Rainfall, average temperature, and relative air humidity after splitting the N fertilization in the 2016/2017 crop season (d); Figure S4: Daily (a) and accumulated (b) N-NH_3 losses by volatilization in slow- and controlled-release N fertilizers. Rainfall, average temperature, and relative air humidity after splitting the N fertilization in the 2016/2017 crop season (c); Figure S5: Accumulated N-NH_3 losses by volatilization in the first, second, and third split application (a, b, and c) of conventional and stabilized N fertilizers in the 2015/2016 crop season; Figure S6: Accumulated N-NH_3 losses by volatilization in the first, second, and third split application (a, b, and c) of conventional and stabilized N fertilizers in the 2016/2017 crop season; Figure S7: Thickness of surface coatings by MEV images (a) and elemental composition of surface coatings (b) of the controlled release source: urea coated with elemental sulfur (S^0) + polymer (Blend N—fertilizer); Figure S8: Thickness of surface coatings by MEV images (a) and elemental composition of surface coatings (b) of the controlled release source: urea coated with plastic resin; Figure S9: Thickness of surface coatings by MEV images (a) and elemental composition of surface coatings (b) of the controlled release source: urea coated with polyurethane; Figure S10: Images of the conventional N fertilizers: urea (a), ammonium nitrate (b), and ammonium sulfate (c); Figure S11: Images of the stabilized N fertilizers: urea treated with NBPT (a) and urea treated with Cu and B; Figure S12: Images of the fertilizers: Slow-release: Urea-formaldehyde (a) and Physical barrier: Urea + adhesive + $CaCO_3$ (b); Figure S13: Dry leaves in the canopy projection of the coffee plant hindering fertilizer incorporation.

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Nitrogen Fertilizers Technologies for Corn in Two Yield Environments in South Brazil

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Abstract: Improvements in nitrogen use efficiency (NUE) in corn production systems are necessary, to decrease the economic and environmental losses caused by loss of ammonia volatilization (NH_3-N). The objective was to study different nitrogen (N) fertilizer technologies through characterization of N sources, NH_3-N volatilization losses, and their effects on the nutrient concentration and yield of corn grown in clayey and sandy soils in south Brazil. The treatments consisted of a control without N application as a topdressing, three conventional N sources (urea, ammonium sulfate, and ammonium nitrate + calcium sulfate), and three enhanced-efficiency fertilizers [urea treated with NBPT + Duromide, urea formaldehyde, and polymer-coated urea (PCU) + urea treated with NBPT and nitrification inhibitor (NI)]. The losses by NH_3-N volatilization were up to 46% of the N applied with urea. However, NI addition to urea increased the N losses by NH_3-N volatilization by 8.8 and 23.3%, in relation to urea alone for clayey and sandy soils, respectively. Clayey soil was 38.4% more responsive than sandy soil to N fertilization. Ammonium sulfate and ammonium nitrate + calcium sulfate showed the best results, because it increased the corn yield in clayey soil and contributed to reductions in NH_3-N emissions of 84 and 80% in relation to urea, respectively.

Keywords: urea; ammonia volatilization; enhanced-efficiency fertilizers; nitrification inhibitor; plant nutrition; X-ray diffraction; scanning electron microscopy

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1. Introduction

Producing food sustainably and sufficiently for humanity has been a challenge over time. At the global level, corn (*Zea mays* L.) is the most produced grain, with 1.2 billion tons produced per year [1], and it will be responsible for a 45% increase in cereal production in the coming years [2], driven by an estimated population expansion to 9.7 billion people by 2050. Although the production potential of corn hybrids has increased through genetic improvements and the development of more technically advanced crops, the world average yield is 5980 kg ha^{-1} [1], far below the productive potential of the crop.

Nitrogen fertilization management is one of the factors that contributes the most to increasing corn yield. In plants, nitrogen (N) is the mineral element required in greatest quantities and is responsible for the synthesis of amino acids, proteins, and enzymes, and for photosynthetic processes [3]. Urea [$CO(NH_2)_2$] is the most commonly used source to meet the N needs of plants, because it has industrial advantages, such as a high N concentration per unit mass (45 to 46%) and lower production costs than other N sources [4]. It is estimated that in 2023, the global demand for N will be 155 Mt yr^{-1}, of which 53% will be supplied by urea [5]. However, once applied to soil, urea is hydrolyzed by the action of urease enzyme, producing ammonia (NH_3-N), which is rapidly lost to the atmosphere in the form of gas [6]. This loss may account for more than 60% of the N applied [7], depending on the soil and air temperature [8], soil moisture [9], soil pH [10], soil buffering capacity [11], presence of straw on the soil surface [12], N source [13], and rate of applied fertilizer N [14].

Although NH_3-N is not a greenhouse gas (GHG), it can indirectly contribute to nitrous oxide (N_2O-N) emissions [15], which are extremely harmful, due to their high global warming potential and permanence in the atmosphere for long periods [16]. Ammonia losses can reduce the N use efficiency (NUE), because less nutrients are left for plant absorption, causing negative yields and economic consequences for farmers [17,18]. In addition, NH_3-N losses in agricultural areas affect air quality and contaminate terrestrial and aquatic ecosystems [19]. In the United States, for example, economic losses of approximately 39 billion dollars and deaths of more than 4300 people annually are linked to air pollution, as a result of NH_3-N emissions from corn production systems that have both low NUE and nitrogen fertilizer overdoses [20].

The incorporation of urea into the soil is an effective way to reduce losses by NH_3-N volatilization and increase NUE. This incorporation can be achieved using mechanical techniques [21] or irrigation [22]. Such practices are not always possible, because less than 20% of the world's areas are irrigated [23] and because they interrupt the no-tillage system, which is an important soil conservation management practice. Therefore, surface application of N is the predominant practice in agricultural production systems. Nitrogen sources such as ammonium sulfate [$(NH_4)_2SO_4$] and ammonium nitrate (NH_4NO_3) are not subject to considerable losses by NH_3-N volatilization [13,24], but tend to be more expensive, due to their lower N concentration. In addition, ammonium nitrate is subject to purchase restrictions by the military, due to its use as an explosive material [4].

To circumvent these limitations, N fertilizer industries have relied on the use of urea as a raw material, due to its high concentration of N for the development of N enhanced-efficiency fertilizers (EEFs), classified as stabilized, slow-release, and controlled-release fertilizers [25]. However, although meta-analysis studies have revealed potential reduction in NH_3-N losses by EEFs in relation to urea of between 39.4 and 52.0%, depending on the soil characteristics and climatic conditions before fertilizer application [26,27], the gains in crop yield are low compared to those obtained with conventional urea, ranging from 5.3 to 6.0% [4,26,27].

The N fertilizer industry has developed new stabilizing molecules to inhibit urease activity and has proposed combinations of enhanced-efficiency technologies, to obtain mixed (two or more granules) and/or complex (single-granule) fertilizers. For example, the new Duromide + N-(n-butyl) thiophosphoric triamide (NBPT) stabilization technology reduced NH_3-N losses by 33% compared to only NBPT [9]. On the other hand, the addition of nitrification inhibitors (NIs), which aims to reduce N_2O-N losses and nitrate leaching (NO_3^--N) [28,29], increases the volatilization of NH_3-N by 35.7% and consequently the indirect emissions of N_2O-N by up to 15.2% [30], leading to major debates on the use of NIs to increase NUE in EEFs [31].

Therefore, the objective of this work was to study the different technologies of N fertilizers through the characterization of their N sources, NH_3-N volatilization losses, and effects on the nutrient concentration and yield of corn grown in clayey and sandy soil in south Brazil.

2. Materials and Methods

2.1. Description of the Sites and Soils

The experiments were conducted in two locations belonging to the Technology Diffusion Unit (UDT) of Cocamar Cooperativa Agroindustrial: one located in the municipality of Floresta (23°35′37″ S; 52°04′06″ W), and another in the municipality of Guairaçá (22°56′48″ S; 52°43′22″ W), at 392 and 478 m above sea level, respectively. The climate of the study area is classified as subtropical humid mesothermal (Cfa) according to the

Köppen-Geiger classification system [32]. The rainfall, temperature, relative air humidity, and irrigation depth data during the experiments are shown in Figure 1.

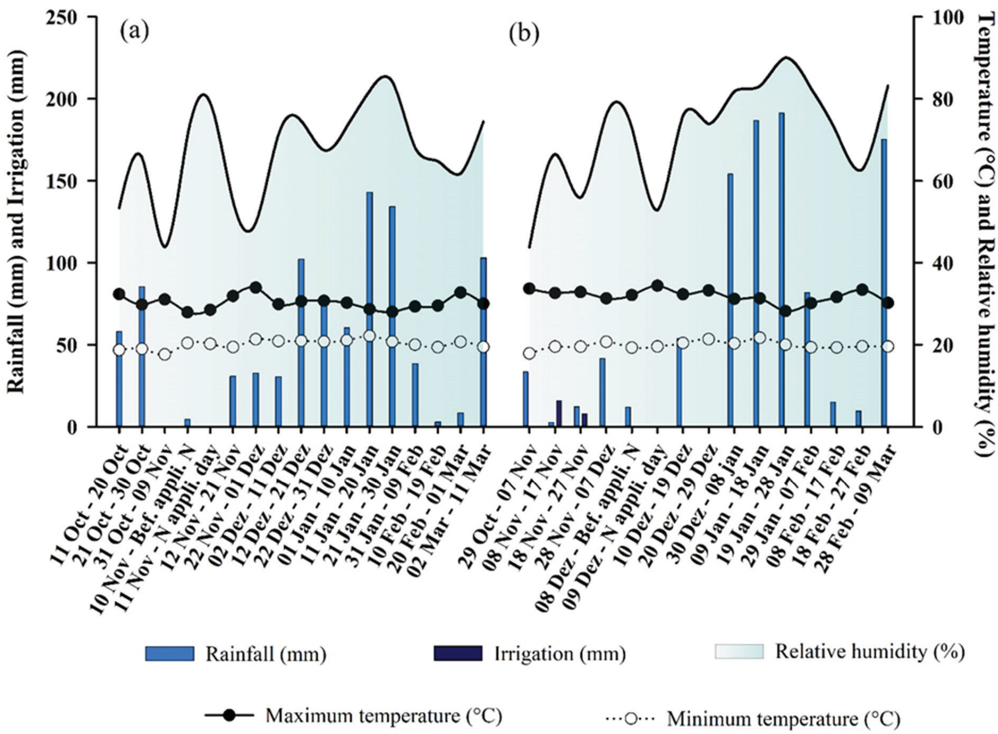

Figure 1. Pluviometric precipitation (mm), air relative humidity (%), maximum and minimum temperature (°C), and irrigation (mm) for corn grown on clayey soil (**a**) and corn grown on sandy soil (**b**). Bef. appli. N: Before application nitrogen day in topdressing. N appli. Day: Day of nitrogen application in topdressing.

The experiments were conducted in the municipalities of Floresta and Guairaçá, located in the state of Paraná, Brazil, in no-till areas, with previous crops of *Brachiaria ruziziensis* and *B. brizantha*, respectively. The soils of the experimental areas in Floresta and Guairaçá were classified as a Latossolo Vermelho distroférrico with clayey texture (clayey soil) and Argissolo Vermelho-Amarelo distrófico (sandy soil) [33], corresponding to an Oxisol and Ultisol, respectively, according to the soil taxonomy of the USDA [34]. Soil samples from the 0–20 m layer were collected for chemical characterization and the determination of particle size (Table 1).

2.2. Experimental Design, Treatments, and Crop Management

A randomized block experimental design was applied, with five replicates and seven treatments. The treatments consisted of a control without N application as topdressing; three conventional nitrogen sources: urea (46% N), ammonium sulfate (21% N and 24% S), and ammonium nitrate + calcium sulfate (27% N, 3.7% S and 5% Ca); and three fertilizers with increased efficiency: one fertilizer stabilized to inhibit the activity of urease enzyme [urea treated with NBPT + Duromide (46% N)], one slow-release fertilizer [urea formaldehyde (37% N)], and one fertilizer consisting of a mixture of granules of different technologies [polymer-coated urea (PCU) (42% N) + urea treated with NBPT and nitrification inhibitor (Ur-NBPT + NI) (46% N) + 3.0% S and 0.3% B in the form of elemental sulfur

(99% S) and ulexite (10% B), respectively]. The experimental units were 4 m wide and 10 m long, yielding a total area of 40 m^2.

Table 1. Chemical and granulometric analysis of an Oxisol (clayey texture), Ultisol (sand texture) and interpretation of values for the surface layer (0.00–0.20 m).

Soil Properties	Clayey Soil [1]	Sand Soil [2]	Soil Attribute
	0.00–0.20 m	**0.00–0.20 m**	**Interpretation [3]**
pH $CaCl_2$	4.50	5.70	Medium [1]/High [2]
H + Al ($cmol_c\ dm^{-3}$)	6.49	1.90	-
Al^{3+} ($cmol_c\ dm^{-3}$)	0.15	0.00	Very low [1,2]
Ca^{2+} ($cmol_c\ dm^{-3}$)	3.31	1.68	High[1]/Medium [2]
Mg^{2+} ($cmol_c\ dm^{-3}$)	1.09	0.52	Medium [1,2]
K^+ ($cmol_c\ dm^{-3}$)	0.17	0.09	Medium [1]/Low [2]
SB ($cmol_c\ dm^{-3}$)	4.57	2.29	-
CEC_{pH7} ($cmol_c\ dm^{-3}$)	11.06	4.19	Medium[1]/Very low [2]
ECEC ($cmol_c\ dm^{-3}$)	4.72	2.29	High[1]/Medium [2]
BS (%)	42	55	Medium[1]/High [2]
P ($mg\ dm^{-3}$)	16.15	56.61	Very high [1,2]
S ($mg\ dm^{-3}$)	2.96	0.95	Medium [1]/Very low [2]
B ($mg\ dm^{-3}$)	0.40	0.12	High [1]/Low [2]
Zn ($mg\ dm^{-3}$)	5.70	12.96	High [1]/Very high [2]
Cu ($mg\ dm^{-3}$)	10.32	5.04	Very high [1,2]
Fe ($mg\ dm^{-3}$)	49.14	25.86	-
Mn ($mg\ dm^{-3}$)	142.26	47.28	Vey high [1]/high [2]
OC ($g\ dm^{-3}$)	16.73	8.26	High [1]/Medium [2]
OM (%)	2.89	1.42	High [1]/Low [2]
Sand (%)	8	89	-
Silt (%)	14	1	-
Clay (%)	78	10	-

pH($CaCl_2$) (0.01 mol L^{-1}) at a soil:solution ratio of 1:2.5; H + Al was determined by the Shoemaker–McLean–Pratt (SMP) method; Ca^{2+}, Mg^{2+}, and Al^{3+} extracted with KCl 1 mol L^{-1}; OM: soil organic matter content obtained by organic carbon × 1.724 (Walkley-Black); P, K^+, Zn, Cu, Fe, and Mn: Mehlich-1 extraction; SO_4^{2-} was extracted by calcium phosphate in acetic acid; B was extracted with hot water; sum of bases (SB): Ca^{2+} + Mg^{2+} + K^+; CEC: cation exchange capacity at pH 7 (SB + H + Al); ECEC: effective cation exchange capacity (SB + Al^{3+}); BS: base saturation [(SB/CEC) × 100]; and particle size distribution (sand, silt, and clay) by densimeter method. [1] Soil attribute interpretation of the clayey soil. [2] Soil attribute interpretation of the sand soil. [3] Interpretation of soil attributes, according to SBCS/NEPAR [35].

Corn (*Zea mays* L.) was sown at the Floresta (clayey soil) and Guairaçá (sandy soil) sites on 14 October and 6 November 2020, under a dry mass cover of 3.15 and 4.76 Mg ha^{-1} *B. ruziziensis* and *B. brizantha*, respectively. The corn hybrids used in Floresta and Guairaçá were Brevant 2433 PWU and FS512 PWU, respectively, with a distribution of 2.7 seeds m^{-1} and a spacing of 0.45 m, totaling 60,000 pl ha^{-1}. Sowing fertilization was performed with the application of 535 and 400 kg ha^{-1} of 10-15-15 (N-P_2O_5-K_2O), and when the corn was at phenological stage V4 (four leaves with collars visible), 60 and 40 kg ha^{-1} K_2O as KCl were applied in Floresta and Guairaçá, respectively. The N fertilizers were applied as a topdressing, according to the expected yield of the Floresta (clayey soil) and Guairaçá (sandy soil) sites at phenological stage V5 (five leaves with collars visible) at doses of 200 and 150 kg ha^{-1}, respectively, as recommended by the Parana State Fertilization and Liming Manual (SBCS/NEPAR) [35].

2.3. Capture and Determination of Ammonia Volatilization

To determine the ammonia volatilization, the N fertilizers were weighed separately with an analytical balance and manually applied in a semi-open static chamber allocated within each experimental unit. For the treatment consisting of a mixture of granules of different technologies, PCU was physically separated from Ur-NBPT + NI, and three chambers were installed in the experimental unit for each treatment; with the first chamber for PCU, the second chamber for Ur-NBPT + NI, and the third chamber for granules mixed at a ratio of 50% PCU and 44% Ur-NBPT + NI, as the product is marketed.

Immediately after the application of N fertilizers in Floresta (clayey soil) and Guairaçá (sandy soil), N losses via ammonia volatilization were quantified through sample collection, performed at 1, 2, 4, 6, 8, 11, 15, 19, 22, 26, 33, 40, 47, 54, 61, 68, 76, and 83 days and 1, 2, 4, 6, 9, 15, 21, 28, 36, 44, 51, 58, 65, 71, and 78 days after application, totaling 18 and 15 collection times, respectively. The chambers were constructed from plastic bottles (polyethylene terephthalate, PET) with a total area of 0.007854 m^2. Each chamber contained a 2.5 cm wide and 25 cm long strip of filter paper with a base immersed in a 50-cm^3 flask with 20 mL 0.05 mol L^{-1} H_2SO_4 and a solution of 2% glycerine (v/v) [36,37]. The used vials were replaced with new vials until the ammonia loss stabilized.

After each collection, the chambers were rotated between the three bases within each experimental unit, to minimize the effects of environmental factors such as rainfall and temperature. Subsequently, the samples were sent to the Soil Fertility Laboratory of the Maringá State University, Paraná, Brazil, and refrigerated until analysis. Ammonia captured in the form of ammonium sulfate was determined by UV/VIS spectrophotometry, using the salicylate green method [37]. During the experimental period of ammonia volatilization sampling, no irrigation was performed.

2.4. Characterization of Nitrogen Fertilizers

The N fertilizers were finely ground and characterized by X-ray diffraction (XRD) analysis (XRD 6000, Shimadzu, Kyoto, Japan). X-ray diffractograms were obtained with a scanning interval of 3° to 70° 2θ, sampling step of 0.02°, and time of 1.2 s using CoKα radiation with a nickel filter (40 kV, 30 mA). The values obtained were exported to X'Pert Highcore Plus software to determine the intensity, peak position, and crystallographic *hkl* plane. To identify the coating layer and coating thickness of the PCU + Ur-NBPT + NI treatment with controlled-release technology, the granules were physically separated into PCU and Ur-NBPT + NI and cut longitudinally with the aid of a scalpel blade. Subsequently, the granules were fixed to a stub microscope support with the aid of carbon tape and then metallized with gold. The samples were then analyzed using scanning electron microscopy (SEM), using a Quanta FEG 250 microscope.

2.5. Evaluation of Nutrient Concentration, Morphological, and Yield Status

During the flowering period of the corn, corresponding to phenological stage R1 (silking), indirect readings of the chlorophyll leaf content (SPAD index) were performed using SPAD 502 Plus® instrument (Konica Minolta, Tokyo, Japan). The nutrient concentration of the corn was evaluated at phenological stage R1, by randomly collecting 15 plants from the middle third of the first leaf opposite and below the upper ear [35]. After collecting the leaves, the samples were sent to the Soil Fertility Laboratory of the Maringá State University, Paraná, Brazil; washed with distilled water; dried in an oven with forced air circulation at 65 °C for 72 h; and ground in a Wiley mill. Subsequently, the samples were weighed and subjected to sulfuric acid and nitric-perchloric acid digestion for the extraction of N, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn). For analysis of leaf boron (B) content, the samples were subjected to dry digestion via calcination in a muffle furnace [38].

The content of Ca, Mg, Zn, Cu, Fe, and Mn were determined by atomic absorption spectrophotometry (AAS) with a mixture of air:acetylene. Phosphorus was determined by vanadate yellow spectrophotometry, S by spectrophotometry using the barium sulfate turbidimetry method, K by flame emission photometry, N by the micro-Kjeldahl method, and B by azomethine-H spectrophotometry [38].

The plant height was determined at phenological stage R2 (milky grains) by measuring from the soil surface to the insertion of the tassel using a tape measure. After the physiological maturation of corn, corresponding to phenological stage R6, manual harvesting of the ears was performed in a useful area of 10.8 m^2, the kernel moisture was corrected to

13%, and the kernel mass of each experimental unit was extrapolated to obtain the yield in kg ha^{-1}.

2.6. Statistical Analysis

The data obtained for corn yield, height, and nutrient concentration were subjected to homogeneity of variance (Bartlett) and error normality (Lilliefors) tests, thus meeting the assumptions of analysis of variance [39]. Subsequently, the data were subjected to joint analysis of variance, provided that the quotient between the largest and smallest residual mean squares of the analysis of individual variances was less than 7 [40]. Treatments and places were considered fixed factors, and their interaction was subdivided into treatments within places and places within treatments ($p < 0.05$), as shown by the follow statistical model. Subsequently, the means were compared using Tukey's test at 5% probability, using the statistical software GENES [41].

$$\mathrm{Y}ijk = \mu + \mathrm{G}i + \mathrm{B/A}jk + \mathrm{A}j + \mathrm{GA}ij + \varepsilon ijk$$

where Y*ijk* is the observed value for treatment *i* (nitrogen fertilizer) in place *j* (clayey or sandy soil) in block *k*; μ is the effect of the mean; G*i* is the fixed effect of treatment i; B/A*jk* is the block nested in place *j*; A*j* is the fixed effect of place *j*; GA*ij* is the interaction between treatment *i* and place *j*; and εijk is the experimental random error in treatment *i*, place *j*, and block *k*.

For the NH_3-N volatilization data, model selection was performed according to the Akaike information criterion (AIC) [42], and the model with the lowest AIC was chosen. After selecting the model, the data were subjected to nonlinear regression using SigmaPlot software, using the logistic model of three parameters (α, β and γ) represented by Equation (1), as described by Seber and Wild [43]. This model has traditionally been used to estimate plant growth and nutrient uptake rates [44] and has been more recently used to estimate losses by NH_3-N volatilization [9,13].

$$\hat{Y} = \frac{\alpha}{1 + \exp^{[-(\mathrm{time}-\beta)/\gamma]}} \quad (1)$$

where $\hat{Y}$ is the amount of N volatilized as NH_3-N (kg ha^{-1}) at time t; α is the maximum accumulated volatilization; β is the time at which a 50% loss occurs, corresponding to the inflection point of the curve (the day when the maximum daily loss of NH_3-N occurs); t is the time (days); and γ is a parameter of the model used to calculate the maximum daily loss (MDL) of NH_3-N, as shown in Equation (2).

$$\mathrm{MDL} = \frac{\alpha}{4\gamma} \quad (2)$$

3. Results

3.1. Scanning Electron Microscopy and X-ray Diffraction

The electron micrographs revealed the transverse morphology of the granules that compose the PCU + Ur-NBPT + NI granule mixture, indicating the presence or absence of coating (Figure 2). Thus, the Ur-NBPT + NI granules do not have a coating layer (Figure 2a), whereas the PCU granules have a polymer coating layer with an average thickness of 34.90 μm (Figure 2b).

The X-ray diffractograms showed typical reflections of the chemical species of each nitrogen fertilizer (Figure 3). The urea-based fertilizers (urea and Ur-NBPT + Duromide) showed characteristic reflections of urea (110) and biuret (Figure 3a,d). The only phase found in ammonium sulfate was the characteristic reflection of this fertilizer (Figure 3b). Conversely, the ammonium nitrate + calcium sulfate-based fertilizer contained dolomite, ammonium nitrate, and calcium sulfate, with more intense *hkl* planes at 104, 111, and 020, respectively (Figure 3c). In Ur-formaldehyde, at least two phases were identified, urea and methylenediurea (MDU), indicated by more intense reflections at 110 and −311, respectively (Figure 3e).

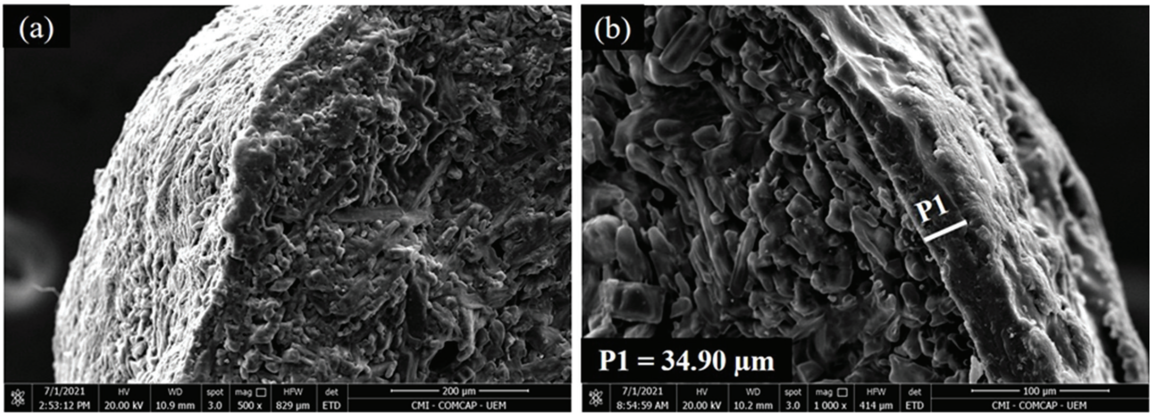

Figure 2. Electron micrographs of the separate Ur-NBPT + NI (**a**) and PCU granules (**b**). P1 is the thickness of the coating layer of the polymer coated granule.

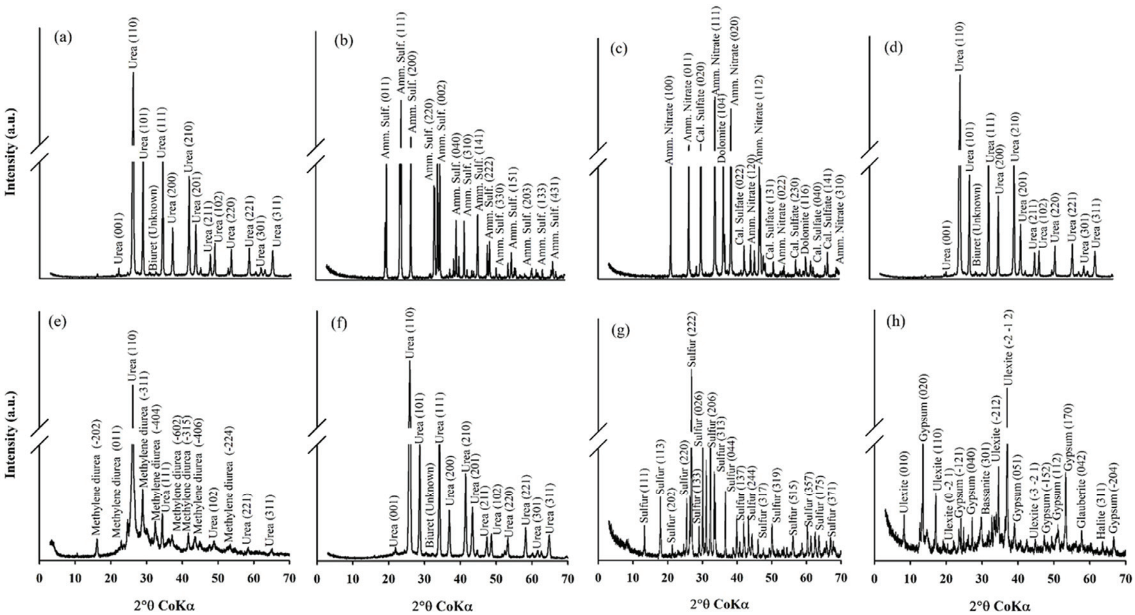

Figure 3. X-ray diffraction of the urea (Ur) (**a**), ammonium sulfate (**b**), ammonium nitrate + calcium sulfate (**c**), Ur-NBPT + Duromide (**d**), Ur-formaldehyde (**e**), PCU + Ur-NBPT + NI (**f**), elemental sulfur (**g**) and ulexite (**h**) for conventional and enhanced efficiency nitrogen fertilizers characterization.

For the mixed fertilizer, the granules were separated into PCU, Ur-NBPT + NI, S granules (elemental sulfur), and B granules (ulexite). The XRD patterns of PCU and Ur-NBPT + NI indicated reflections characteristic of urea and biuret (Figure 3f). The S granule was identified as elemental sulfur with a more intense plane at 222 (Figure 3g). The B granule showed several phases, such as ulexite, gypsum, glauberite halite, and bassanite, with more intense planes at -2-12, 020, 311, 042, and 301, respectively (Figure 3h).

3.2. N Losses through Ammonia Volatilization

The climatic conditions before the application of the N topdressing fertilizers are shown in Figure 1. The fertilizers were applied 24 h after 4.6 and 12 mm rainfall to the clayey and sandy soil, respectively. The maximum and minimum temperatures during the first 76 h after fertilization were 31.1 and 19.9 °C, respectively, for Floresta (clayey soil) and 34.0 and 20.2 °C for Guairaçá (sandy soil). The volatilization of NH_3-N followed a sigmoidal pattern, increasing at the beginning, reaching the maximum daily loss, and then stabilizing (Figure 4). The maximum accumulated loss (α) of NH_3-N according to the adjusted model decreased in the following order: urea (41.0 and 69.2 kg ha^{-1} NH_3-N; 20.5 and 46.1% of the N applied), PCU + Ur-NBPT + NI (40.1 and 62.5 kg ha^{-1} NH_3-N; 20.0 and 41.7% of the N applied); Ur-NBPT + Duromide (33.4 and 44.5 kg ha^{-1} NH_3-N; 16.7 and 29.7% of the N applied), Ur-formaldehyde (18.3 and 30.5 kg ha^{-1} NH_3-N; 9.1 and 20.3% of the N applied), ammonium nitrate + calcium sulfate (10.7 and 13.6 kg ha^{-1} NH_3-N; 5.3 and 9.1% of the N applied), and ammonium sulfate (8.5 and 11.0 kg ha^{-1} NH_3-N; 4.2 and 7.3% of the N applied) for clayey and sandy soils, respectively (Table 2).

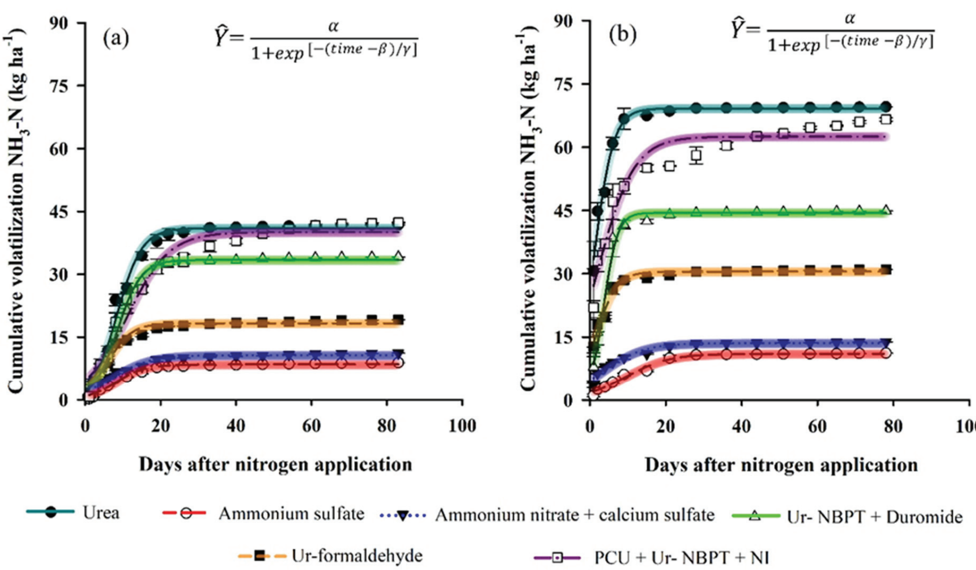

Figure 4. Cumulative volatilization of NH3-N after topdressing applications of urea, ammonium sulfate, ammonium nitrate + calcium sulfate, Ur-NBPT + Duromide, Ur-formaldehyde, and PCU + Ur-NBPT + NI for clayey soil at a rate of 200 kg ha^{-1} of N (**a**) and for sand soil at a rate of 150 kg ha^{-1} of N (**b**). Data with overlapping vertical bars with 95% confidence interval in the curve.

Ammonium sulfate, ammonium nitrate + calcium sulfate, Ur-formaldehyde, Ur-NBPT + Duromide, and PCU + Ur-NBPT + NI reduced NH_3-N losses by 79.3 and 84.1, 73.9 and 80.3, 55.4 and 55.9, 18.5 and 35.7, and 2.2 and 9.7% compared to urea for clayey and sandy soil, respectively (Table 2). Ammonium sulfate and ammonium nitrate + calcium sulfate were the sources that most reduced the NH_3-N volatilization losses in both locations compared to urea, with average reductions of 81.7 and 77.1%, respectively. The peak NH_3-N volatilization (β) of urea, ammonium sulfate, ammonium nitrate + calcium sulfate, Ur-NBPT + Duromide, Ur-formaldehyde, and PCU + Ur-NBPT + NI occurred at 8.4 and 1.2, 8.3 and 7.1, 7.3 and 3.5, 8.5 and 3.6, 6.0 and 1.6, and 11.1 and 2.2 days after application of topdressing fertilizers to clayey and sandy soil, respectively (Table 2).

Table 2. Nonlinear regression parameters adjusted (logistic model) for NH_3-N volatilization cumulative losses for conventional and enhanced efficiency nitrogen fertilizers and reduction of NH_3-N emission in relation to urea for clayey and sandy soils.

Treatments	Soil and Rate at Topdressing	Parameters				MDL	Reduction of Losses of NH_3-N in Relation to Urea (%)
		α kg ha^{-1} NH_3-N	γ	β day	R^2	kg ha^{-1} day^{-1} NH_3-N	
Urea		41.0	3.3	8.4	0.98	3.10	-
Amm. sulfate		8.5	3.8	8.3	0.97	0.56	79.3
Amm. nitrate + Cal. sulfate	Clayey	10.7	5.0	7.3	0.96	0.54	73.9
Ur-NBPT + Duromide	(200 kg ha^{-1})	33.4	3.1	8.5	0.98	2.69	18.5
Ur-formaldehyde		18.3	3.2	6.0	0.96	1.43	55.4
PCU + Ur-NBPT + NI		40.1	5.5	11.1	0.96	1.82	2.2
Urea		69.2	2.5	1.2	0.97	6.92	-
Amm. sulfate		11.0	5.8	7.1	0.98	0.47	84.1
Amm. nitrate + Cal. sulfate	Sandy	13.6	5.3	3.5	0.95	0.64	80.3
Ur-NBPT + Duromide	(150 kg ha^{-1})	44.5	1.8	3.6	0.97	6.18	35.7
Ur-formaldehyde		30.5	2.7	1.6	0.97	2.82	55.9
PCU + Ur-NBPT + NI		62.5	4.7	2.2	0.93	3.32	9.7

α: maximum cumulative volatilization; β: time at which 50% of the losses occur, corresponding to the curve inflection point; γ: parameter of the equation used to calculate the MDL (maximum daily loss of NH_3-N).

The environment in the sandy soil area provided lower β compared to the environment in the clayey soil area, and the NH_3-N volatilization peaks advanced by 8.9, 7.2, 4.9, 4.4, 3.8 and 1.2 days for PCU + Ur-NBPT + NI, urea, Ur-NBPT + Duromide, Ur-formaldehyde, ammonium nitrate + calcium sulfate, and ammonium sulfate, respectively. The maximum daily loss (MDL) of NH_3-N decreased in the following order: urea (3.10 and 6.92 kg ha^{-1} NH_3-N), Ur -NBPT + Duromide (2.69 and 6.18 kg ha^{-1} NH_3-N), PCU + Ur-NBPT + NI (1.82 and 3.32 kg ha^{-1} NH_3-N), Ur-formaldehyde (1.43 and 2.82 kg ha^{-1} NH_3-N), ammonium nitrate + calcium sulfate (0.54 and 0.64 kg ha^{-1} NH_3-N), and ammonium sulfate (0.56 and 0.47 kg ha^{-1} NH_3-N) for clayey and sandy soil, respectively (Table 2). The greatest reductions in MDL were obtained with the use of ammonium sulfate and ammonium nitrate + calcium sulfate in both locations.

3.3. Ammonia Volatilization in Granules with and without Nitrification Inhibitor

According to the fitted model (Figure 5), the maximum accumulated losses of NH_3-N for the granules decreased in the order Ur -NBPT + NI (44.6 and 85.3 kg ha^{-1} NH_3-N; 22.3 and 56.9% of the N applied) followed by PCU (40.3 and 44.9 kg ha^{-1} NH_3-N; 20.1 and 29.9% of the N applied) for clayey and sandy soil, respectively (Table 3). Urea-NBPT + NI granules increased the NH_3-N volatilization losses by 8.8 and 23.3% compared to urea for clayey and sandy soils, respectively.

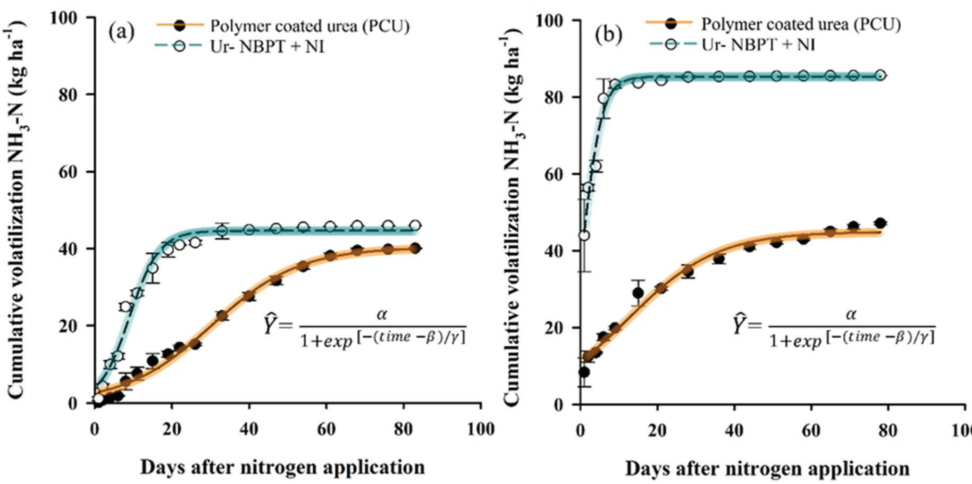

Figure 5. Cumulative volatilization of NH3-N after topdressing applications of the separate granules PCU and Ur-NBPT + NI for clayey soil at a rate of 200 kg ha^{-1} of N (**a**) and for sand soil at a rate of 150 kg ha^{-1} of N (**b**). Data with overlapping vertical bars with 95% confidence interval in the curve.

The time of peak NH_3-N volatilization of the granules occurred in the order PCU (30.6 and 12.6 days) followed by Ur-NBPT + NI (9.0 and 0.7 days), corresponding to delays in the peak of NH_3-N volatilization of 21.6 and 11.9 days with PCU compared to Ur-NBPT + NI for clayey and sandy soil, respectively (Table 3). The MDL of Ur-NBPT + NI was 2.93 and 8.53 kg ha^{-1} NH_3-N, followed by PCU with 0.89 and 0.98 kg ha^{-1} NH_3-N for clayey and sandy soil, respectively (Table 3). The fertilizer Ur-NBPT + NI increased MDL by 69.6 and 88.5% relative to PCU for clayey and sandy soil, respectively.

Table 3. Nonlinear regression parameters adjusted (logistic model) for NH_3-N volatilization cumulative losses of the separate granules PCU and Ur-NBPT + NI and the increase of NH_3-N emission in relation to urea for clayey and sandy soils.

Treatments	Soil and Rate at Topdressing	Parameters				MDL	Increased of Losses of NH_3-N in Relation to Urea (%)
		α kg ha^{-1} NH_3-N	γ	β day	R^2	kg ha^{-1} day^{-1} NH_3-N	
PCU	Clayey	40.3	11.3	30.6	0.98	0.89	-
Ur-NBPT + NI	(200 kg ha^{-1})	44.6	3.8	9.0	0.98	2.93	8.8
PCU	Sandy	44.9	11.5	12.6	0.97	0.98	-
Ur-NBPT + NI	(150 kg ha^{-1})	85.3	2.5	0.7	0.97	8.53	23.3

α: maximum cumulative volatilization; β: time at which 50% of the losses occur, corresponding to the curve inflection point; γ: parameter of the equation used to calculate the MDL (maximum daily loss of NH_3-N).

3.4. Leaf Macronutrient Content in Corn

Regarding the leaf contents of macronutrients (Figure 6), the application of nitrogen fertilizers in topdressing increased leaf N contents by 6.31 (23.6%) and 3.05 (11.0%) g kg^{-1} in comparison to the control for the clayey and sandy soils, respectively. However, there was no difference between the nitrogen sources in either soils. The results for nitrogen fertilizers applied as a topdressing in clayey soil were 2.12 (6.9%) g kg^{-1} higher than those for the same applications performed in sandy soil, with the exception of the control, which showed no differences in leaf N content between the clayey and sandy soils (Figure 6a). The nitrogen sources increased the levels of leaf P and K only in a clayey soil environment, with a mean increase in relation to the control of 1.02 (37.9%) and 1.68 (11.7%) g kg^{-1}, respectively, and no differences between the sources. All treatments, with or without application of N in topdressing, conducted in sandy soil had higher levels of leaf P and K than those conducted in clayey soil, with a mean difference of 1.35 (37.9%) and 4.78 (29.2%) g kg^{-1}, respectively (Figure 6b,c).

In the clayey soil, corn without N fertilization as a topdressing had a higher leaf Ca content than the PCU + Ur-NBPT + NI, ammonium sulfate, and ammonium nitrate + calcium sulfate treatments, with increases of 1.22 (33.5%), 1.31 (36.9%), and 1.45 (42.5%) g kg^{-1}, respectively. There was no significant difference between treatments for leaf Ca levels in corn grown in sandy soil and no significant differences between locations (Figure 6d). Regarding the levels of leaf Mg, in clayey soil, ammonium nitrate + calcium sulfate and the control had higher levels than ammonium sulfate, with differences of 0.76 (34.8%) and 0.71 (37.3%) g kg^{-1}, respectively. There was no significant difference between treatments regarding leaf Mg content in the corn grown in sandy soil. Regarding the locations, the values for ammonium nitrate + calcium sulfate and the control in clayey soil were 0.44 (19.0%) and 0.48 (20.7%) g kg^{-1} higher than the corresponding values in sandy soil, respectively (Figure 6e).

The ammonium sulfate source provided the highest leaf S levels in corn in both clayey and sandy soil, with an average increase of 0.55 (21.9%) and 0.85 (38.5%) g kg^{-1} for clayey soil and 0.27 (12.0%) and 0.50 (24.7%) g kg^{-1} for sandy soil compared to the other nitrogen sources and the control, respectively. Although ammonium sulfate provided the largest increases in leaf S, the sources urea, ammonium nitrate, Ur-NBPT + Duromide, Ur-formaldehyde, and PCU + Ur-NBPT + NI also increased the levels of leaf S, but only in relation to the control, with average values of 0.30 (13.6%) and 0.23 (24.7%) g kg^{-1} for clayey and sandy soils, respectively. Corn, with or without application of N as a topdressing, grown in clayey soil had, on average, 0.29 (12.8%) g kg^{-1} more leaf S than corn grown in sandy soil (Figure 6f).

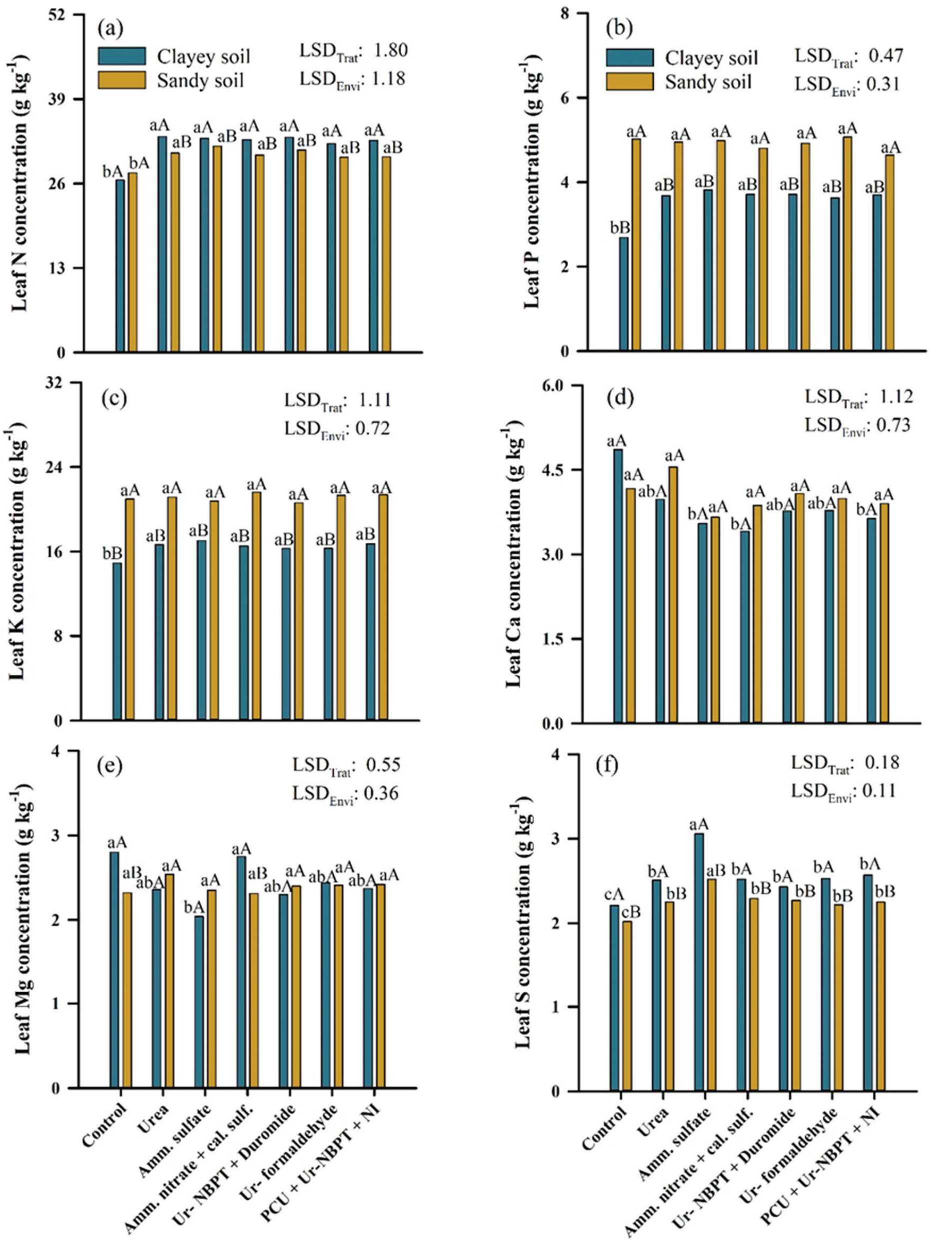

Figure 6. Concentration of nitrogen (**a**), phosphorus (**b**), potassium (**c**), calcium (**d**), magnesium (**e**), and sulfur (**f**) in the corn leaf after fertilization in topdressing with nitrogen sources urea, ammonium sulfate, ammonium nitrate + calcium sulfate, Ur-NBPT + Duromide, Ur-formaldehyde, and PCU + Ur-NBPT + NI for clayey and sand soil. Treatments followed by the same lowercase letter do not differ using a Tukey test ($p < 0.05$). Environments followed by the same capital letter do not differ using a Tukey test ($p < 0.05$). LSD_{Trat}: least significant difference for treatments. LSD_{Envi}: least significant difference for environment.

3.5. Leaf Micronutrient Content and SPAD Index

Regarding the micronutrient contents (Figure 7), leaf Fe and Mn were not influenced by N fertilization as a topdressing for either the clayey or sandy soil. However, the levels of leaf Fe and Mn in corn cultivated in a clayey soil environment were higher, at 55.32 (47.6%) and 263.99 (313.9%) mg kg^{-1}, than the values for corn cultivated in sandy soil, respectively (Figure 7a,b). For leaf Zn, there was no difference between treatments in clayey soil. However, in the ammonium sulfate treatment in sandy soil, the leaf Zn content increased in relation to the control by 7.34 (38.1%) mg kg^{-1}. All treatments, with or without application of N as a topdressing, conducted in clayey soil were higher to those conducted in sandy soil, with a mean difference of 19.46 (88.5%) mg kg^{-1} in leaf Zn (Figure 7c).

Leaf Cu levels increased with topdressing N fertilization only in relation to the control, with an average increase of 4.13 (40.1%) mg kg^{-1} for clayey soil; but in sandy soil, there was no difference between treatments. Similarly to Fe, Mn, and Zn, the levels of leaf Cu in corn grown in a clayey soil environment were also higher than those in corn grown in a sandy soil environment, with a mean difference of 5.16 (59.5%) mg kg^{-1} in leaf Cu (Figure 7d). The application of PCU + Ur -NBPT + NI increased the leaf B content in both the clayey and sandy environments; but in clayey soil, an increase of 3.18 (30.9%) mg kg^{-1} of B occurred only in relation to the control, while in the sandy soil, an increase of 3.41 (32.9%) and 4.46 (45.0%) mg kg^{-1} in leaf B occurred in relation to the other nitrogen sources and the control, respectively. There were no significant differences in leaf B content between clayey and sandy soil (Figure 7e).

Regarding the indirect chlorophyll content (SPAD), the application of N fertilizers as topdressing increased the SPAD index by 11.58 (20.2%) and 5.27 (11.2%) compared to the control for clayey and sandy soil, respectively. However, there was no difference between the nitrogen sources in either production environment. The SPAD indexes of all treatments, with or without application of N as topdressing, conducted in clayey soil were higher than those in sandy soil, with a mean difference of 15.67 (30.4%) (Figure 7f).

3.6. Yield and Height of Corn Plants

Nitrogen fertilizers increased the corn yield only in a clayey soil environment. Increases in yield were obtained with the use of ammonium sulfate, PCU + Ur-NBPT + NI and ammonium nitrate + calcium sulfate, which resulted in increases of 1722 (20.6%), 1838 (21.9%), and 2088 (24.9%) kg ha^{-1} corn, respectively, compared to the control that did not receive N fertilization as a topdressing. All treatments, with or without application of N as topdressing, conducted in clayey soil were higher than those conducted in sandy soil, with a mean difference in yield of 3654 kg ha^{-1}, equivalent to 37.4%. However, when considering only the treatments that received N as a topdressing, the difference in yield became 370 kg ha^{-1}, equivalent to 38.4% (Figure 8a). Topdressing N fertilization did not influence plant height in either the clayey or sandy soil environment. However, the plant height in clayey soil was 23 cm higher on average than that in sandy soil (Figure 8b).

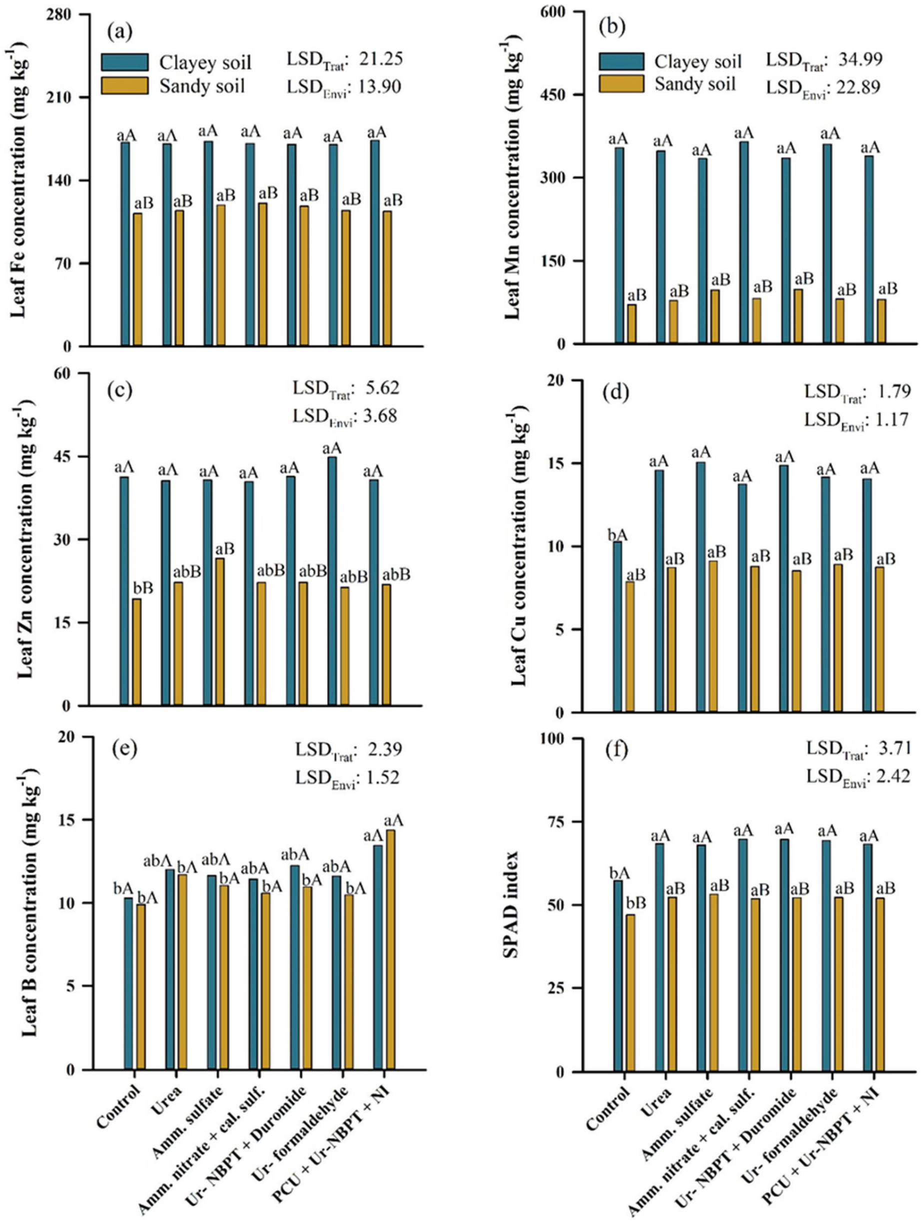

Figure 7. Concentration of iron (**a**), copper (**b**), zinc (**c**), manganese (**d**), boron (**e**), and SPAD index (**f**) in the corn leaf after fertilization in topdressing with nitrogen sources urea, ammonium sulfate, ammonium nitrate + calcium sulfate, Ur-NBPT + Duromide, Ur-formaldehyde, and PCU + Ur-NBPT + NI for clayey and sand soil. Treatments followed by the same lowercase letter do not differ using a Tukey test ($p < 0.05$). Environments followed by the same capital letter do not differ using a Tukey test ($p < 0.05$). LSD$_{Trat}$: least significant difference for treatments. LSD$_{Envi}$: least significant difference for environment.

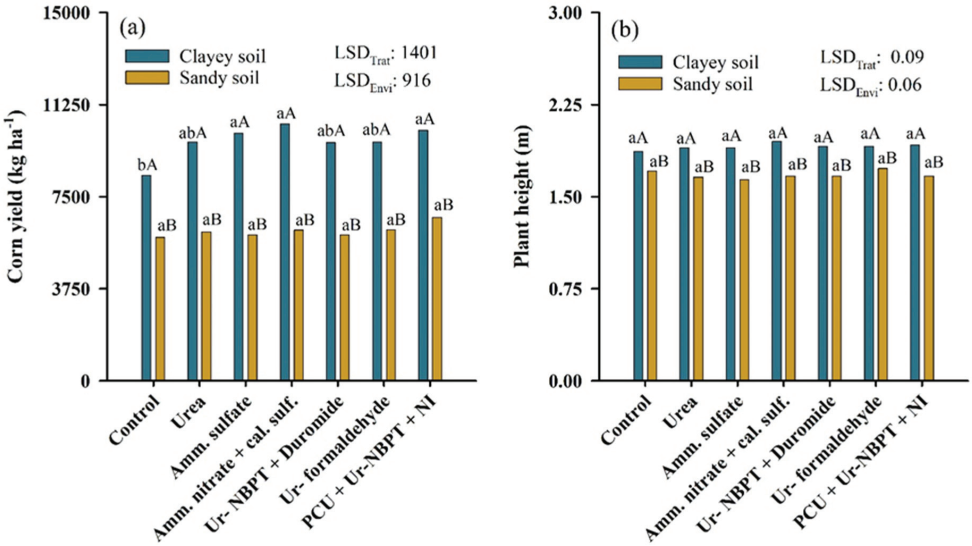

Figure 8. Corn yield (**a**) and plant height (**b**) after fertilization in topdressing with nitrogen sources urea, ammonium sulfate, ammonium nitrate + calcium sulfate, Ur-NBPT + Duromide, Ur-formaldehyde, and PCU + Ur-NBPT + NI for clayey and sand soil. Treatments followed by the same lowercase letter do not differ using a Tukey test ($p < 0.05$). Environments followed by the same capital letter do not differ using a Tukey test ($p < 0.05$). LSD_{Trat}: least significant difference for treatments. LSD_{Envi}: least significant difference for environment.

4. Discussion

4.1. X-ray Diffraction and SEM of N Fertilizers

The industrial production of N fertilizers in amidic form (Figure 3a,d,f) produces biuret as a by-product, resulting from the increase in temperature above the melting point of urea, which is 132 °C [45]. Although biuret is a common impurity, Brazilian legislation allows only up to 2% in solid N fertilizer [46], because it is a toxic chemical compound that interferes with the protein synthesis of plants [47]. Currently, biuret toxicity is insignificant in crops, due to advances in the technology used to manufacture urea fertilizers [48]. Although Ur-formaldehyde comes from an amidic source, biuret was not found in the XRD analysis, probably due to strict controls in the production process.

Dolomite, which was identified in the X-ray diffractogram of ammonium nitrate + calcium sulfate (Figure 3c), inhibits the exothermic and undesirable decomposition of ammonium nitrate, thus improving the safety of the fertilizer [49]. According to standard NFPA 490 of the National Fire Protection Association [50], ammonium nitrate is not considered flammable or combustible. However, factors such as a high temperatures under confinement (260 to 300 °C) and contamination by organic or inorganic materials, such as chlorides or powdered metals, can lead to explosive detonation with the production of nitrous oxide, which is decomposed into nitrogen and oxygen [51,52].

Urea-formaldehyde was the first synthetic nitrogen fertilizer with low solubility to be marketed for slow release of N. The production process consists of condensation within a reactor with controlled pH, temperature, molar ratio, and reaction time between urea and formaldehyde [25]. The final product of the reaction consists of a mixture of methylene urea polymers (methylene urea, MDU and polymethylene) with differences in the degree of polymerization (insolubility) and molecular weight (chain length) [53,54]. Thus, the presence of MDU in Ur-formaldehyde (Figure 3e) will provide an intermediate molecular weight and degree of polymerization, contributing to the slow release of N. This compound, combined with certain amounts of unreacted urea, results in an intelligent fertilizer for agricultural use within the cultivation time of the plants. For example, Cassim et al. [55] observed no increase in yield in cultures with the application of Ur-formaldehyde with 70% slow-release compounds; but, with proportions of 55 and 60% of slow-release compounds, the yield increases were significant.

The lack of sulfur in the mixture of PCU + Ur-NBPT + NI granules (Figure 3f) indicates that the coating layer of the PCU granules (Figure 2b) is covered only by polymer and not by elemental sulfur (S^0). This configuration provides better nutrient release kinetics than an S^0 coating, because it is independent of the activity of microorganisms responsible for the oxidation of S^0 [54]. However, the production cost of PCU is higher. The source of B present in the PCU + Ur-NBPT + NI + B + S mixture was identified as ulexite, an evaporite formed under arid conditions in saline lakes supported by hydrothermal vents and linked to volcanic activity [56]. For this reason, ulexite may be associated with other evaporites, such as halite, gypsum, glauberite, and bassanite, as described in Figure 3h.

4.2. Ammonia Volatilization of N Sources in Clayey and Sandy Soil

The losses by NH_3-N volatilization were higher in sandy soil for all N sources tested (Figures 4 and 5). This behavior occurred due to the higher moisture content of the sandy soil, resulting from the higher rainfall volume 24 h before the application of the nitrogen fertilizers as a topdressing (Figure 1). Under dry soil conditions, the urease hydrolysis rate is low; however, the rate increases as the soil water content increases [57]. Above 20% moisture, hydrolysis is practically no longer affected by changes in soil moisture [4].

The previous considerations explain the sigmoidal behavior of NH_3-N volatilization losses, which depend on the increase in urease enzyme activity [58], which consumes the H^+ resulting from urea hydrolysis, as demonstrated by the reaction $CO(NH_2)_2 + 2H^+ + 2H_2O \rightarrow 2NH_4{}^+ + H_2CO_3$ [59]. This reaction promotes the increase in soil pH around N fertilizer granules to approximately 8.7, changing the balance between $NH_4{}^+$ and NH_3 [60]. After reaching the maximum loss (α), NH_3-N emissions decrease over time, due to the gradual reduction in pH and stabilization of N in the form of $NH_4{}^+$-N [24].

In addition to differences in soil moisture, the clay content and, consequently, the cation exchange capacity (CEC) (Table 1) are the main differences between the two soil classes studied that will also influence the intensity of NH_3-N volatilization. Clayey soils with a higher CEC have more exchange sites to retain the $NH_4{}^+$ produced in the hydrolysis of urea due to adsorption. In addition, the higher buffering capacity of soils with a higher CEC provides greater resistance to changes in soil pH around N granules caused by the urease enzyme, thus decreasing the intensity of NH_3 -N volatilization [58,59]. Therefore, the lower loss of NH_3-N by volatilization in clayey soils resulted in higher inflection points in the NH_3-N curve (β) and a lower MDL, as described in Tables 2 and 3.

Ammonium sulfate and ammonium nitrate + calcium sulfate had the lowest accumulated losses of NH_3-N in relation to the other N sources in both production environments (Figure 4), due to the absence of N in the amidic form (NH_2-N). Corrêa et al., Minato et al., and Otto et al. [13,14,24] also obtained low losses due to NH_3-N volatilization with the use of N sources in the ammoniacal and nitric forms, with losses ranging from 0.7 to 5.2% and 1.0 to 7.7% of the N applied for ammonium sulfate and ammonium nitrate, respectively, depending on the dose of applied topdressing. Following the increasing order of NH_3-N emissions, Ur-formaldehyde was the enhanced-efficiency source that most reduced NH_3-N volatilization, but it was less efficient than ammoniacal and nitric sources. Although Ur-formaldehyde can reduce the solubility of N fractions through the synthesis of methylene urea groups, it contains some urea that does not react with formaldehyde (Figure 3e), favoring losses by volatilization of NH_3-N, even in small proportions.

The next formulation considered is Ur-NBPT + Duromide, a stabilizer that combines two NBPT + Duromide molecules, both of which inhibit the activity of the urease enzyme, but having the advantage of a more stable chemical structure under low pH and high soil temperature conditions [9]. However, the use of Ur-NBPT + Duromide showed higher emissions of NH_3-N (mean of 23.5% of the applied N) when compared to meta-analysis studies that obtained losses by volatilization of NH_3-N of 14.8% using NBPT [26]. Under conditions with large amounts of straw on the soil surface, such as those described in the present study (mean of 3.95 Mg ha^{-1} Brachiaria straw), the amount of urease enzyme in the soil will be higher, increasing losses by NH_3-N volatilization by up to 25.5% [7]. In other words, the stabilizers can reduce, but not eliminate, the activity of the urease enzyme, possibly due to the high amounts of this enzyme in systems with high amounts of straw. Thus, such materials are not the most appropriate technology for production environments with large amounts of straw residues on the soil surface.

The mixture of PCU + Ur-NBPT + NI was inefficient in reducing losses by NH_3-N volatilization, with losses very close to those of conventional urea (Figure 4 and Table 2). As PCU + Ur-NBPT + NI is a mixed fertilizer, composed of different granules, the granules were separated to understand the efficiency of each component in reducing or contributing to NH_3-N emissions (Figure 5). Although PCU granules are designed to release N at a controlled rate to synchronize with the crop demand and reduce environmental pollution by NO_3^--N, NH_3-N and N_2O-N [61], factors such as high temperatures, excessive rainfall, the number and thickness of the coating layers, and quality of the coating material may have interfered with the efficiency of PCU, contributing to the release of N in the amidic form and losses in the form of NH_3-N.

On the other hand, the addition of NI to Ur-NBPT to mitigate direct emissions of N_2O-N [28] and losses by leaching of NO_3^--N [62] was the factor that most contributed to the inefficiency of the mixture PCU + Ur-NBPT + NI, since the addition of NI to NBPT significantly decreased the ability of NBPT to inhibit urea hydrolysis by up to 21% [63], in addition to contributing to the increase in NH_3-N volatilization losses [30,31].

4.3. Nitrification Inhibitor Increases Losses Due to Ammonia Volatilization

The results showed that the use of NI significantly increased the volatilization of NH_3-N relative to the PCU granules, especially in sandy soil (Figure 5b). According to Wu et al. [30], there are two main mechanisms associated with increased volatilization: (i) NIs are a group of chemical compounds that inhibit the activity of *Nitrosomonas* spp. bacteria responsible for the oxidation of NH_4^+ to nitrite (NO_2^-) and, therefore, increase the soil concentration of NH_4^+ that is converted to NH_3; and (ii) NIs induce a liming effect. Qiao et al. [64] found that the application of NI increased soil pH by 0.23 units, due to the decelerated rate of nitrification and increased efficiency of N use by plants resulting from the lower leaching of NO_3^-. Thus, unleached NO_3^- is absorbed by plant roots, which excrete OH^- to maintain the electrochemical balance in the soil, thus increasing the pH of the medium [64,65]. Once the soil pH changes, the balance between NH_3 and NH_4^+ is affected; as the soil pH increases, the equilibrium shifts, leading to the transformation of NH_3-N and its subsequent loss to the atmosphere in the form of gas [60].

The volatilization of NH_3-N can be influenced by several factors, such as dose, N source, climatic conditions, management system, and soil attributes, with the latter being the main factor responsible for altering the efficiency of NIs. For example, a meta-analysis performed by Kim et al. [66] found that treatments with NI increased emissions of NH_3-N in soils with higher pH (5.4 to 7.9) and smaller ranges of CEC (5.7 to 16.8 $cmol_c\ dm^{-3}$) in comparison with lower-pH soils (4.7 to 6.2) and larger CEC ranges (10.0 to 24.0 $cmol_c\ dm^{-3}$). This effect occurs due to the favored formation of NH_3-N at basic pH, combined with soils of low CEC, which provide fewer exchange sites for NH_4^+ adsorption, facilitating the loss of N by volatilization [67].

Another important soil attribute is the organic matter (OM) content. Soils with high levels of OM have higher amounts of *Nitrosomonas* spp., which hinders the performance of NIs [29] and leads to a need for higher concentrations of NI in soils with high OM content. In addition, high clay contents will favor lower emissions of NH_3-N, due to their contribution to increasing soil CEC [68]. This explains the higher volatilization of urea treated with NI in sandy soil, since the pH, OM, CEC, and clay content were 4.50 and 5.70, 2.89 and 1.42%, 11.06 and 4.19 $cmol_c\ dm^{-3}$, and 78 and 10% for the clayey and sandy soils, respectively (Table 1). Therefore, the use of NIs, especially in sandy textured soils in rainfed agriculture, is not recommended as a strategy to increase the NUE. The INs technology in nitrogen fertilizers is more efficient in flooded agriculture systems, due to the denitrification losses of N_2O-N and N_2, representing up to 34% of the applied N [69].

4.4. Nitrogen Sources and Nutrient Concentration of Corn—Macronutrients

Although there were differences in losses due to NH_3-N volatilization between N sources, the N applied as topdressing fertilization that was not lost by volatilization may have been sufficient to meet the N demand of the corn crop. As a result, changes in the concentration N status were not observed between the N sources, but only in relation to the control that did not receive N as topdressing (Figure 6a). According to Cantarella et al. [4], in many cases, most of the N absorbed by crops comes from soil OM, and fertilizer N, although important to increase yield, provides complementary N. Similarly, Oliveira et al. [70], working with ^{15}N isotopes, observed that only 33% of the N absorbed by corn plants was derived from topdressing nitrogen fertilization.

Topdressing N fertilization was important for the maintenance of leaf chlorophyll content, which was indirectly quantified by the increase in SPAD index in relation to the control in both production environments (Figure 7f). According to Taiz et al. [3], chlorophylls are green photosynthetic pigments that have a porphyrin-like ring structure with a Mg atom coordinated in the center, linked to four other N atoms, with a long tail of hydrocarbons. Thus, in the absence of N, the plant degrades chlorophyll molecules to obtain the four N atoms that are part of its structure, developing generalized chlorosis in the leaf and compromising the absorption of light.

The highest leaf N concentration and chlorophyll content (SPAD) being in the corn grown in clayey soil was due to the higher expected yield provided by the better fertility of clayey soils than sandy soils, requiring a higher photosynthetic rate [71], and the higher N uptake by the plants, since each ton of corn grains requires 21.5 kg of N [35]. This effect was not observed in the accumulation of leaf N among the controls, most likely due to the lack of fertilization as topdressing, which inhibited the realization of the productive potential of the clayey soil environment.

After N, K is the nutrient most absorbed by corn plants, followed by P. Thus, the increase in cultivation intensity and, thus, higher yields obtained through N fertilization provided greater absorption of K and P and consequently a higher accumulation of these nutrients in the leaf (Figure 6b,c). Since P is a key element for the synthesis of molecules such as DNA, RNA, ATP, and NADPH [72], and as K is important for the activation of enzymatic systems and protein synthesis [73], synergistic interactions exist between N $\times$ P and N $\times$ K [74]. For example, Rietra et al. [75] performed a meta-analysis on the interaction between nutrients and found a synergism between N $\times$ P and N $\times$ K and no case of antagonism, in a total of 77 studies. The highest leaf concentration of P being in corn grown in sandy soil resulted from a lower adsorption to iron and aluminum oxides, which has a positive correlation with clay content [76]. Higher concentrations of leaf K were also observed in corn grown in sandy soil due to the low CEC of the soil, providing lower adsorption of K^+ and consequently greater availability in the soil solution.

Elements are absorbed at different rates, due to their affinity with membrane carriers, obeying the decreasing cationic order $NH_4^+ > K^+ > Na^+ > Mg^{2+} > Ca^{2+}$ [38]. Thus, because Ca is absorbed by the roots in the form of Ca^{2+}, its absorption may be compromised by the high concentrations of NH_4^+ in the soil solution, due to competition [77]. Therefore, the highest accumulation of leaf Ca being in the control cultivated in clayey soil, relative to the values obtained with ammonium sulfate, ammonium nitrate + calcium sulfate, and PCU + Ur-NBPT + NI (Figure 6d), occurred due to two factors: (i) high doses of fertilizers containing NH_4^+; and (ii) the use of fertilizers with NIs. The use of NIs will inhibit the nitrification process, increasing the level of NH_4^+ in the soil, thus suppressing the absorption of Ca^{2+} and resulting in a lower accumulation in leaves. This effect was also observed in the reduction in leaf Mg levels in corn grown in clayey soil using ammonium sulfate compared to the control (Figure 6e). Thus, high concentrations of NH_4^+ can also reduce the absorption of Mg^{2+} and K^+ by plants [78–80].

However, unlike Ca, the use of ammonium nitrate + calcium sulfate promoted an increase in leaf Mg, due to the presence of dolomite in its composition (Figure 3c), which inhibits the undesirable exothermic decomposition process of ammonium nitrate [49], in addition to being an important source of Mg^{2+} to plants [81]. On the other hand, reductions in leaf K accumulation were not observed with the use of ammoniacal sources, because the suppression is greater with divalent cations (Ca^{2+} and Mg^{2+}) than with monovalent cations (K^+) [82]. Regarding the differences between the production environments, the control conducted in sandy soil obtained lower concentrations of leaf Mg because the soil content was below the critical level (Table 1), which was 1 $cmol_c$ dm^{-3} [35]. For ammonium nitrate + calcium sulfate, the highest concentration of leaf Mg in corn grown in clayey soil resulted from the highest dose of nitrogen application in topsoil, which thus provided more Mg in the form of dolomite.

Many plant compounds, such as amino acids and proteins, have both N and S, which helps explain the existence of a positive N/S ratio and the increases in leaf S concentration in both corn production environments with N application as topdressing compared to the control (Figure 6f). However, ammonium sulfate was the N source that provided the highest leaf S concentrations, due to the high concentration of S per unit mass (24%) in the form of sulfate (SO_4^{2-}), which is the main form absorbed by plants and does not require oxidation by *Thiobacillus*, which, in turn, is dependent on soil temperature and moisture conditions [83]. On the other hand, the sandy soil probably favored more intense leaching of SO_4^{2-} due to the few anionic adsorption sites, decreasing the contact with the root system and, consequently, absorption by the plant [84]. This mechanism explains the higher concentration of S in the leaves of corn grown in clayey soil, as described in Figure 6f.

4.5. Nitrogen Sources and Nutrient Concentration of Corn—Micronutrients

The nitrogen fertilizers did not alter the leaf concentrations of Fe and Mn. However, the leaf concentrations varied between the production environments. The natural levels of micronutrients in soil depend on the chemical composition of the parent material, pedogenetic processes, and the degree of soil weathering [85]. Thus, the parent material of the clayey soil located in northern Paraná state is basalt, an igneous rock rich in micronutrients such as Fe and Mn, because these elements have the same geochemical formation environments [86]. In contrast, the sandy soil located in the northwestern part of the Paraná state originates from sandstones of the Caiuá Formation, a sedimentary rock whose main constituent mineral is quartz [87]. Consequently, higher levels of Fe and Mn are naturally available in clayey soil, favoring greater absorption and concentration of these nutrients in corn leaves (Figure 7a,b).

The use of nitrogen fertilizers in agriculture promotes the production of H^+ through the oxidation of ammonium to nitrate, as demonstrated by the nitrification reaction $NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$ [88]. With a reduction in soil pH, the availability of metal cationic micronutrients increases [38], providing higher leaf concentrations of Zn in corn grown in sandy soil (Figure 7c) and Cu in corn grown in clayey soil (Figure 7d). However, the highest concentrations of leaf Zn occurred with the application of ammonium sulfate, which is an exclusively ammoniacal source, and the soil acidification process was intensified because more NH_4^+ was provided as a substrate for nitrifying bacteria [89]. An increase in leaf Zn with the use of ammonium sulfate was not observed in clayey soil, due to the higher CEC and OM content, which favored the acidity buffering reaction through the exchange of H^+ ions for basic cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) in clay minerals and OM [90], which is the predominant buffering mechanism in the pH range between 4.2 and 5.0 [91].

Regarding the differences in Zn levels between the production environments, the higher grain yield obtained in clayey soil (Figure 8a) required a higher Zn absorption by the plants, because Zn is the most exported micronutrient in corn, with 24.8 g for each ton of grain produced [35]. Cooper, in turn, is the micronutrient that interacts the most with soil organic compounds and forms stable complexes, especially with carboxylic and phenolic groups of OM, in addition to having a strong affinity for clay [92]. Therefore, sandy soils, with low OM levels, are mostly deficient in Cu, due to leaching losses. This explains the higher concentration of leaf Cu in clayey soils than in sandy soils (Figure 7d).

The presence of ulexite in the PCU + Ur-NBPT + NI mixture identified by XRD (Figure 3h) resulted in an increase in leaf B concentration in both corn production environments (Figure 7e). However, soluble B sources such as borax ($Na_2B_4O_7 \cdot 10H_2O$) and boric acid (H_3BO_3) are more commonly used to maintain plant growth when compared to lower-solubility sources such as ulexite ($NaCaB_5O_9 \cdot 8H_2O$) and colemanite ($Ca_2B_6O_{11} \cdot 5H_2O$) [93]. However, the ulexite present in the PCU + Ur-NBPT + NI mixture is of acid origin and is obtained through the granulation process with the use of sulfuric acid, which provides an increase in water solubility of approximately 90%. This higher solubility favors the faster release of B, making the nutrient available for plant absorption and increasing leaf B concentrations.

4.6. Clay Soils Are More Responsive to Nitrogen Fertilization

The sandy soil, because it originated from the Caiuá sandstone formation, is characterized by low a CEC, due to its high sand content, especially coarse sand [87]. A low CEC directly affects cation losses, due to leaching, and consequently the expected crop yield. The low fertility of sandy soils was confirmed by the lower growth of corn plants in the sandy soil than in the clayey soil (Figure 8b). Thus, in a production environment with a low response to fertilization, topdressing nitrogen fertilization contributes less to increases in corn yield (Figure 8a). In a meta-analysis performed by Tremblay et al. [94], the authors concluded that corn yield increased by a factor of 1.6 in sandy soils and 2.7 in clayey soils after nitrogen fertilization, showing that corn is more responsive to N fertilization in clay soils.

In addition to the influence of CEC on the response to nitrogen fertilization, soil texture can play a role. For example, clay affects the stabilization of organic N through the protection of OM by aggregates, favoring the preservation of microbial biomass [95]. Ros et al. [96] studied the variation in mineralizable N and its relationship with physical properties in 98 agricultural soils in the Netherlands and observed a lower N mineralization rate in clayey soils than in sandy soils. Ping, Ferguson, and Dobermann [97] found that corn needed less N fertilizer in sandy soils than in clayey soils. This may suggest that soil texture influences the degree of OM stabilization and, consequently, the response to nitrogen fertilization, increasing the chances of an increased yield via N fertilization in clayey soils.

Soil texture also provides different degrees of water storage in the soil. Therefore, sandy soils, due to their higher porosity, store less water, resulting in higher metabolic costs to the plant to absorb water and promote transpiration, which can consequently affect the yield. Although the response to nitrogen fertilization is lower in sandy soils than soils with other textures, studies of fertilization with varying sources and doses of N in these locations should be performed, mainly because such soils are highly susceptible to losses by NH_3-N volatilization and because they are the main soils of new agricultural frontiers in Brazil [98,99].

The lower losses by NH_3-N volatilization obtained with the use of ammonium sulfate and ammonium nitrate + calcium sulfate increased the corn yield in clayey soil. With a reduction in NH_3-N losses, N will be used more efficiently by the plant, favoring the synthesis of biomolecules essential for corn growth and development. However, other characteristics of these N sources may also have influenced the yield and should be mentioned. For example, the higher concentration of leaf S provided by the application of ammonium sulfate may have contributed to the increase in corn yield, because S is closely linked to N metabolism, converting nonprotein N into protein [83]. Thus, all plant metabolism depends on S compounds, due to the structural functions they perform, such as maintaining the active conformation of proteins through the disulfide bonds between methionine and cysteine (S-S) and metabolic functions, since they constitute amino acids, coenzymes, and proteins with Fe and S [3,100].

Not only did ammonium nitrate + calcium sulfate generate a low loss by NH_3-N volatilization, but synergistic benefits for plant growth have been observed if NO_3^- and NH_4^+ are provided together [80,101]. The beneficial effect of the simultaneous supply of these two inorganic forms of N occurs due to the lower suppression of the absorption of cationic nutrients, mainly Ca^{2+} and Mg^{2+}, by the exclusive supply of NH_4^+ [80], lower acidification or alkalinization of the rhizosphere as a result of the absorption in excess of NH_4^+ or NO_3^- [65], and lower energy requirements for NH_4^+ assimilation than NO_3^- assimilation, given that NO_3^- cannot be used directly by plants until it is reduced to NH_4^+; a reduction catalyzed sequentially by nitrate reductase enzymes and nitrite reductase [102,103].

The N source PCU + Ur -NBPT + NI also increased the corn yield in clayey soil. However, this effect was not caused by a reduction in NH_3-N volatilization, but by the supply of B via ulexite and synchronization of N release with PCU. Boron is responsible for plant functions such as sugar translocation and the regulation of carbohydrate and phytohormone metabolism. However, B plays a very important role in the metabolism of N. This is due to the requirement of B for the synthesis of the uracil nitrogen base, an essential component of RNA, which is also indispensable for ribosome formation and protein synthesis [38]. Therefore, an increased availability of B in soil favors higher yields, mainly because it is a nutrient found in low concentrations in tropical and subtropical soils, due to losses by leaching in the form of $H_3BO_3^0$.

Even given the inefficiency of PCU granules in reducing losses by NH_3-N volatilization (Figure 5a), in most cases, controlled-release technology can decrease the availability of N at the beginning of corn development, when the absorption is still low [27], and increases the availability of N in phenological stages VT (tasseling) to R1, when the demand for N by corn is high [104]. This behavior in the later release stages of N was observed through the maximum daily loss of NH_3-N in PCU granules alone (β), which occurred 30.6 days after topdressing fertilization in the clayey soil (Table 3); with enough time for the corn to be in VT, which usually coincides with the eighth week after emergence [105].

5. Conclusions

The losses by NH_3-N volatilization were up to 46% of the N applied with urea. However, NI addition to urea increased N losses by NH_3-N volatilization by 8.8 and 23.3% in relation to only urea for clayey and sandy soils, respectively. This leads to important implications for the use of NI as a mitigation tool for climate change in rainfed agriculture.

The nitrogen fertilizer technologies applied in a topdressing on clayey and sandy soil presented the following, in decreasing order, losses by volatilization of NH_3-N: urea > URP + Ur-NBPT + IN > Ur-NBPT + Duromide > Ur-formaldehyde > ammonium nitrate + calcium sulfate > ammonium sulfate.

Soil with a clayey texture was 38.4% more responsive to nitrogen fertilization than soil with a sandy texture. The increase in corn grain yield in the clayey soil did not occur only due to the reduction in losses by NH_3-N volatilization, other factors, such as S and B supplementation and N release at a controlled rate, to synchronize with the crop demand, also influenced the increase in corn yield. However, it is always advisable to choose N sources that increase crop yield, while generating the lowest possible losses due to NH_3-N volatilization. In this study, these sources were ammonium sulfate and ammonium nitrate + calcium sulfate, which contributed to reductions in NH_3-N emissions of 84 and 80% in relation to urea, respectively, thus favoring more profitable and sustainable agriculture.

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Improvement of Photosynthesis by Biochar and Vermicompost to Enhance Tomato (*Solanum lycopersicum* L.) Yield under Greenhouse Conditions

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Abstract: Chlorophyll fluorescence is an important tool in the study of photosynthesis and its effect on the physiological indicators of crop growth is worth exploring. The trial was conducted to investigate the effect of biochar (CK, 0%; BA_3, 3%; BA_5, 5%; by mass of soil) and vermicompost (VA_3, 3%; VA_5, 5%) on photosynthesis, chlorophyll fluorescence, and tomato yield under greenhouse condition. Results revealed that photosynthetic parameters and chlorophyll fluorescence traits of BA_3, VA_3, BA_5, and VA_5 were significantly higher than those of CK, and the improvement of vermicompost was more effective than biochar at the same application rate. VA_3 treatment had the highest net photosynthetic rate (*Pn*), intercellular CO_2 concentration (*Ci*), variable fluorescence (*Fv*), maximum fluorescence (*Fm*), *PSII* maximum photochemical efficiency (*Fv/Fm*), *PSII* potential photochemical activity (*Fv/Fo*), absorption flux per cross section (CS; *ABC/CSm*), trapped energy flux per CS (*TRo/CSm*), and electron transport flux per CS (*ETo/CSm*), which increased by 49%, 65%, 17%, 12%, 4%, 25%, 10%, 15%, and 30%, respectively, compared with CK. The study also found that BA and VA rates could effectively improve tomato yield and water use efficiency (*WUE*). The yield under BA_3, VA_3, BA_5, and VA_5 treatments was 21%, 33%, 23%, and 25% higher than that under CK, and the *WUE* increased from 31.2 $kg \cdot m^{-3}$ under CK to 41.4 $kg \cdot m^{-3}$ under VA_3. Pearson correlation analysis indicated that the increment of photosynthesis showed a highly significant correlation with *Fv/Fo*, *ABC/CSm*, *TRo/CSm*, and *ETo/CSm* and enhanced the light energy absorbed, trapped, and transported per CS of plant leaves, thereby contributing to the increase in tomato yield. Therefore, for one-season tomato production, the application of 3% vermicompost was considered economical with regard to improving photosynthesis, enhancing *WUE*, and increasing tomato yield.

Keywords: biochar; vermicompost; net photosynthetic rate; intercellular CO_2 concentration; photosystem II; maximum photochemical efficiency; active reaction center density

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1. Introduction

As an effective way to improve vegetable production, facility cultivation plays an important role in anti-seasonal and inter-regional vegetable cultivation in China [1]. Tomato is a temperature-loving, light-loving, and semi-drought-tolerant facility cultivation crop with high promotion potential and economic value [2]. However, unreasonable fertilizer application and cultivation management have resulted in facility soils showing a susceptibility to pests and diseases, which seriously affected the growth and development of tomatoes and reduced tomato yield [3,4]. Therefore, optimizing the fertilizer application pattern and improving the photosynthesis and chlorophyll fluorescence characteristics of plants are important for the high yield and quality of tomatoes.

Photosynthesis is the basic physiological activity for crop yield formation, and the strength of photosynthesis is closely related to the level of yield [5–7]. Photosynthesis is influenced by various environmental factors, including water, nutrients, light, and CO_2

concentration [8–10]. Biochar is beneficial to plant growth and physiological characteristic indicators, and it can improve the growth performance and yield of different crops [11]. Appropriate application of biochar can improve the apparent quantum efficiency, *Pn*, photosynthetic capacity, and stomatal conductance (*Gs*) of plants [12], which can play a role in improving crop quality and yield [13]. Zhu [14] showed that biochar increased *Pn* and seedling emergence and promoted plant height and dry matter mass of tomatoes. Cao et al. [15] found that biochar improved the nutritional quality of cherry tomato fruits and increased the yield. Lu et al. [16] concluded that biochar could increase the chlorophyll content of plant leaves and positively affect crop yield. The special physical and chemical properties and biological structure of vermicompost improve the transpiration rate (*Tr*), *Gs*, *Pn*, and intercellular CO_2 concentration (*Ci*) of tomato leaves, thereby promoting photosynthesis in plants [17]. Hosseinzadeh et al. [18] showed that the application of vermicompost could improve the photosynthesis of the crop primarily because it increased the CO_2 content of the crop roots and improved the soil water-holding capacity. In addition, numerous studies have demonstrated that the application of vermicompost in soil had a positive effect on crop growth and yield [19]. Zhou et al. [20] showed that the application of 80% vermicompost in soil significantly increased the growth of height and stem diameter of tomato plants. Joshi et al. [21] revealed that the application of 45% vermicompost also promoted the growth and development of tomato plants.

Geng et al. [22] concluded that chlorophyll fluorescence parameters could reflect the absorption and conversion of light energy, energy transfer, distribution, and photosynthesis in plants, which were important indicators used to study plant stress resistance physiology and increase crop yield. Chlorophyll fluorescence can be used to study the effect of environmental changes on the photosynthetic structure of plant photosystem II (*PSII*) and yield responses to the efficiency of light energy conversion in plants [23,24]. Li et al. [25] revealed that biochar treatment could significantly affect the chlorophyll fluorescence parameters of cucumber, but the application of 0.5–2.0% biochar had no significant effect on the maximum photochemical efficiency (*Fv/Fm*). Meanwhile, Cheng et al. [26] also revealed that *Fv/Fm* and the actual quantum yield of *PSII* (*Y (II)*) of plant leaves significantly increased when the vermicompost content was higher than 50%. Gong et al. [27] found that *Fv/Fm* of *PSII* and the actual photochemical efficiency (*ΦPSII*) of plants were elevated by water–nitrogen content, and a moderate increase in nitrogen fertilization could improve *Fv/Fm* and *ΦPSII* of crop leaves, enhance crop growth traits, and increase yield.

Numerous studies have shown that the application of biochar and vermicompost changed the physicochemical properties of soil and microbial communities. It also affected the physiological and biochemical properties of plants. Furthermore, photosynthesis has become an important indicator of tomato production, which is essential for promoting plant growth and development and improving yield. Therefore, the tomato water use efficiency (*WUE*) together with plant physiology must be improved through the use of accurate soil management methods, with emphasis on the methods that improve soil quality by the application of biochar and vermicompost with a considerable enhancement in plant physiological responses and yield. To date, there has been little knowledge on the interactive effects of biochar and vermicompost application on photosynthesis rate, especially using the chlorophyll fluorescence of tomato as a probe. Additionally, the effects of biochar and vermicompost on synergistic response of plant growth are not well understood. Moreover, data on the synergistic response of tomato yield with *WUE* are largely scarce [28]. Thus, this study hypothesized that increasing biochar and vermicompost addition could improve the soil properties; regulating the plant photosynthesis by improving chlorophyll fluorescence parameters would, thus, improve the tomatoes' productivity. The study also assumed that the biochar and vermicompost application to the plant can increase plant growth, contributing to an increasing yield of tomato. To test the hypothesis mentioned above, the study investigated the effects of increasing biochar and vermicompost amendment application rates under greenhouse conditions on soil properties related to plant growth. The study also measured the photosynthesis and chlorophyll fluorescence of tomato by measuring the

Pn, *Tr*, *Gs*, *Ci*, including *Fo*, *Fv*, *Fm*, *Fv/Fm*, *Fv/Fo*, *ABC/CSm*, *RC/CSm*, *ETo/CSm*, *DIo/CSm*, and *TRo/CSm* and, thus, regulated tomato yield in a greenhouse experiment to provide proper regulation for the high quality and yield of tomatoes under greenhouse conditions.

2. Materials and Methods

2.1. Experimental Site

Greenhouse experiments were carried out from 25 July 2020 to 11 January 2021 in a non-temperature-controlled greenhouse under natural light conditions, at the water-saving Park of Hohai University located at latitude 31°57′ N and longitude 118°50′ E, at 144 m above sea level in Jiangning District, Nanjing City, Jiangsu Province, China. The climate of the region is humid subtropical, and it is influenced by the East Asian monsoon. The average annual temperature in the region was 15.7 °C; the absolute maximum temperature reached 40.4 °C in August 2020, and the absolute minimum temperature dropped to −13.3 °C in January 2021. The rainy season spanned from July to September, and the average annual rainfall in the area was nearly 1025.12 mm, which was concentrated in the rainy–summer season. The annual sunshine time was 2200 h, and the annual average evaporation was approximately 900 mm. The average monthly rainfall and temperature in the greenhouse during the years of the experiment (2020–2021) are shown in Table 1.

Table 1. Mean monthly climate records during the years of the experiment (2020–2021).

Season	**2020**						**2021**
Month	Jul	Aug	Sept	Oct	Nov	Dec	Jan
Max. temp °C	33.8	40.1	30.3	29.3	16.4	9.8	8.3
Min. temp °C	23.4	29.2	17.7	14.8	8.3	2.1	1.2
Max. Relative humidity %	98.6	85.0	89.5	76.0	82.3	82.6	80.2
Min. Relative humidity %	69.5	62.4	68.7	68.9	70.1	70.8	69.1
Sunshine (h)	10.2	11.4	9.1	8.3	6.5	6.1	5.8
Solar Rad. MJ m^{-2} day^{-1}	97.8	113.7	78.2	73.8	58.7	56.0	55.4

Note: Meteorological data were monitored using HOBO mini weather stations.

2.2. Soil, Biochar, and Vermicompost Preparation

The experimental soil was collected from the top 10–20 cm of the farmland soil of the water-saving Park of Hohai University (31°57′ N, 118°50′ E) and classified as a typical yellow–brown loam based on the Chinese classification [29]. The tested biochar was classified as maize straw biochar (purchased from Henan Lize Environmental Protection Technology Co., Ltd., Zhengzhou, China), and the experimental vermicompost was obtained by fermenting pure cow dung through the digestive system of earthworms. The physicochemical properties of the soil before the experiment, biochar, and vermicompost are shown in Table 2.

The physicochemical properties of the abovementioned soil samples, biochar, and vermicompost were measured by the following methods: Available potassium was determined using flame photometry [30]. Available nitrogen was determined by using a UV–Vis spectrophotometer (L007, 7522112059A; Essence Technology Instruments, Shanghai, China) [31,32]. Available phosphorus was measured using UV–Vis spectrophotometry [33]. Organic matter content was determined using high-temperature oxidation [34]. pH value was determined by using the Remag pH meter in 1:5 samples and water extracts [35].

Table 2. Specific physicochemical properties of soil, biochar, and vermicompost.

Property	Soil	Biochar	Vermicompost
BD	1.41 g·cm^{-3}	0.42 g·cm^{-3}	-
TP	46.3%	55.3%	-
FC	28.7%	-	-
K	101 mg·kg^{-1}	58,513 mg·kg^{-1}	1892 mg·kg^{-1}
N	11.1 mg·kg^{-1}	390 mg·kg^{-1}	564 mg·kg^{-1}
P	5.81 mg·kg^{-1}	56.4 mg·kg^{-1}	461 mg·kg^{-1}
OMC	1.04%	41.1%	44.9%
pH	7.07 value	9.40 value	8.17 value

Note: Values are the average of three replicates of each property; *BD*, *TP*, FC, *K*, *N*, *P*, and *OMC* indicate bulk density, total porosity, field capacity, available potassium, available nitrogen, available phosphorus, and organic matter content, respectively.

2.3. Greenhouse Experimental Setup

The main treatments used biochar and vermicompost. This experiment included the following five treatments: CK (0% rate, 12 kg soil + no addition), BA_3 (3% rate, 12 kg soil + 360 g biochar), VA_3 (3% rate, 12 kg soil + 360 g vermicompost), BA_5 (5% rate, 12 kg soil + 600 g biochar), and VA_5 (5% rate, 12 kg soil + 600 g vermicompost) on a mass basis. Each pot (cylindrical, top diameter: 32.5 cm, bottom diameter: 28 cm, height: 38.5 cm) was filled with quartz sand to a height of 8 cm, considering the water permeability and air permeability of the roots. The soil was air-dried and passed through a 6.3 mm sieve, and then biochar and vermicompost were weighed and mixed with soil thoroughly in proportion, respectively, and added to the pot with a natural bulk density based on each treatment. The pots were placed in a non-temperature-controlled greenhouse under natural light conditions and arranged in a completely randomized block design. Each treatment was replicated eight times (Figure 1).

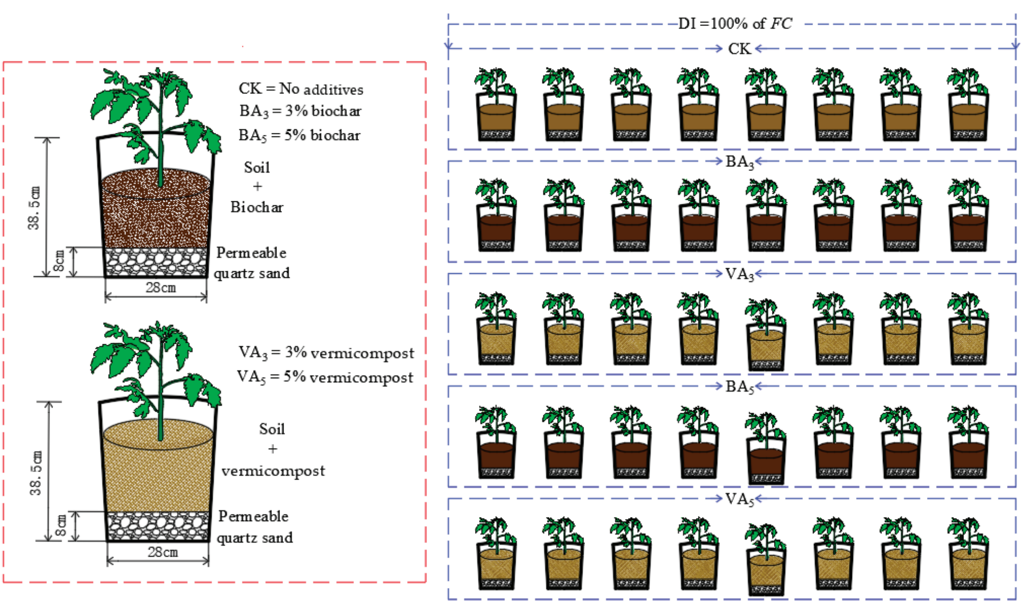


Figure 1. Layout of tomato pots in the greenhouse.

The experimental tomato variety was "Cooperative 903," which is a widely cultivated vegetable planted in Jiangsu Province, China. Tomato seeds were sown at a density of 2–3 seeds per hole in a 72-hole plate of cultivation seedlings and diluted to one plant per well after 2 weeks of seed germination. When seedlings developed five leaves and a heart, seedlings of similar growth were selected and transplanted into pots on 25 August 2020. The tomatoes were managed uniformly based on the experience of local agronomic practices. That is, each pot was applied with 20 g of compound fertilizer (N:P:K = 15:15:15) as a base fertilizer. Each pot was irrigated with tap water to maintain the soil water content at *FC*. The irrigation of each pot was carried out in accordance with the difference in daily weight to compensate for the water loss caused by evaporation [36], and the soil moisture content of all pots was maintained at *FC* throughout the experimental period. Each pot was frequently weeded by hand, and four fruits were left on each inflorescence in each treatment, leaving three leaves pinched at the top after the second inflorescence had set fruit. In addition, field management was carried out in the greenhouse to control pests and diseases and avoid yield losses. The final harvest was completed in January 2021.

2.4. Measurement Items and Methods

2.4.1. Determination of Photosynthetic Parameters

During flowering and fruit setting of the experiment, four plants were randomly selected for each treatment. Healthy fully expanded leaves with sufficient light exposure and consistent leaf position and without visible symptoms of damage at the first inflorescence were selected from each plant. The net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), transpiration rate (*Tr*), and intercellular CO_2 concentration (*Ci*) were determined using a portable photosynthesis system (Li-6800, LI-COR, Lincoln, NE, USA) under an artificial light source with a radiation flux density of 1000 μmol·m^{-2}·s^{-1} from 9:00 a.m. to 11:00 a.m. on a sunny day. The limitation of stomatal conductance (*Ls*) was calculated using the following equation [37]:

$$Ls = 1 - \frac{Ci}{Ca} \tag{1}$$

where *Ls* is the limitation of stomatal conductance; *Ci* is the intercellular CO_2 concentration; *Ca* is the ambient CO_2 concentration.

2.4.2. Determination of Chlorophyll Fluorescence Traits

Chlorophyll fluorescence parameters of plant leaves were measured using a portable chlorophyll fluorometer (Pocket PEA, Hansatech, King's Lynn, UK), and the measurement time and plant site were the same as the photosynthetic parameters. After the leaves were subjected to dark-adapted treatment for 20 min, the rapid chlorophyll fluorescence induction kinetic curve (O-J-I-P curve) was measured using Pocket PEA, which was induced by 5000 μmol·m^{-2}·s^{-1} of pulsed light, and the fluorescence signal was recorded from 10 μs to 2 s. The initial rate of recording was 105 datapoints per second, and the initial fluorescence (*Fo*), maximum fluorescence (*Fm*), and fluorescence intensity at 2 ms of the O-J-I-P curve (F_J) were obtained. Fluorescence parameters were calculated as follows [38,39]: variable fluorescence ($Fv = (Fm - Fo)/Fm$), *PSII* maximum photochemical efficiency (*Fv/Fm*), and *PSII* potential photochemical activity (*Fv/Fo*). In addition, the tomato leaf energy partitioning ratio and *PSII* reaction center activity parameters were measured as follows: absorption flux per cross section ($ABC/CSm \approx Fm$), trapped energy flux per CS ($TRo/CSm = (1 - Fo/Fm)\cdot(ABC/CSm)$), electron transport flux per CS ($ETo/CSm = (1 - Fo/Fm)\cdot(1-(F_J - Fo)/(Fm - Fo)\cdot(ABC/CSm)$), non-photochemical quenching per CS ($DIo/CSm = (ABC/CSm) - (TRo/CSm)$), and the number of active reaction centers per CS (*RC/CSm*) [40].

2.4.3. Determination of Tomato Yield and WUE

At the mature stage of tomato, the yield of four plants selected for each treatment was measured. Fruits of two inflorescences were collected sequentially on the basis of their ripeness and weighed on an electronic scale with an accuracy of 0.01 g. Then, the total fresh weight of fruits of these two inflorescences was calculated as the total yield of each plant. The yield was converted on the basis of planting density (45,000 plants·hm^{-2}). The WUE was determined using the following equation [41]:

$$WUE = \frac{Y}{TWU} \tag{2}$$

where WUE is the water use efficiency, kg·m^{-3}; Y is the tomato yield, kg·hm^{-2}; and TWU is the total water use, m^3·hm^{-2}.

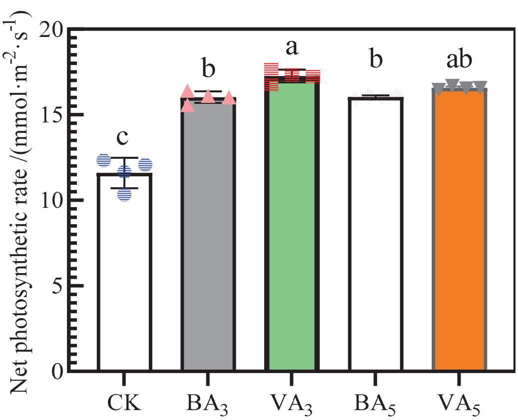
2.5. Data Processing and Analysis

Experimentally measured data were recorded and analyzed by Excel 2010 and one-way analysis of variance (ANOVA) and plotted by GraphPad Prism 8.0. ANOVA was performed using SPSS 26.0, where Duncan's multiple range test was used to compare data means at the 0.05 level of significance, and statistical significance was considered when $p \leq 0.05$. Pearson correlation analysis was also conducted to obtain the degree of relationship among photosynthetic parameters, chlorophyll fluorescence traits, and tomato yield.

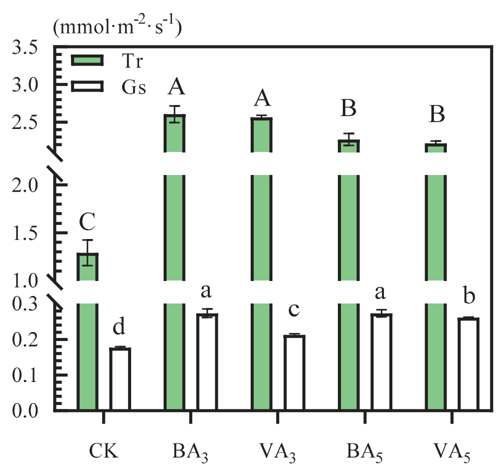
3. Results and Analysis

3.1. Net Photosynthetic Rate and Photosynthetic Parameters

The measured plant photosynthetic parameters during flowering and fruit setting of the experiment were significantly ($p < 0.05$) affected by biochar and vermicompost application (Figure 2). *Pn* increased significantly with the increase in BA and VA rates (Figure 2a), in which VA_3 treatment had the highest *Pn*, with an increase of 49% compared with CK. In addition, VA_3 was significantly different from BA_3 and BA_5 ($p < 0.05$), and no significant difference was observed between BA_3 and BA_5 treatments.



(**a**)



(**b**)

Figure 2. *Cont.*

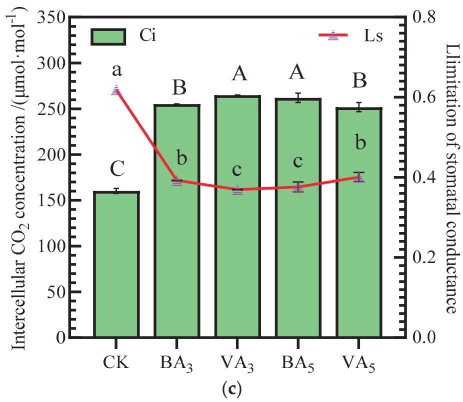


Figure 2. Effects of BA and VA rates on photosynthetic parameters of tomato leaves: (**a**) Effects of BA and VA rates on the *Pn*. (**b**) Effects of BA and VA rates on the *Tr* and *Gs*. (**c**) Effects of BA and VA rates on the *Ci* and *Ls*. Note: BA represents biochar application and VA vermicompost application. The meanings of the circles, triangles, and rectangles on the bars in (**a**) are indicated as duplicate data points for the different treatments. Means of *Pn*, *Gs*, and *Ls* are significantly different between BA and VA rates ($p \leq 0.05$) when followed by different lowercase letters. Means of *Tr* and *Ci* are significantly different between BA and VA rates ($p \leq 0.05$) when followed by different uppercase letters.Meanwhile, the increase in *Pn* in BA_3, VA_3, BA_5, and VA_5 was accompanied by a decrease in *Ls* and an increase in *Ci*, *Tr*, and *Gs*. As shown in (**b**), the highest and lowest T_r rates were observed for BA_3 and CK treatments, respectively, and BA_3 and VA_3 showed no statistically significant difference. G_s under BA_3 treatment was the highest, whereas that under CK treatment was the lowest. For C_i (**c**), the highest C_i was observed for treatments under VA_3, followed by plants under BA_5, BA_3, and VA_5, whereas the lowest values were observed under CK. L_s under BA_3, VA_3, BA_5, and VA_5 treatments decreased by 37%, 40%, 39%, and 35%, respectively, compared with that under CK treatment.

3.2. Chlorophyll Fluorescence Traits

The important chlorophyll fluorescence parameters of *Fo*, *Fv*, *Fm*, *Fv/Fm*, and *Fv/Fo* for different biochar and vermicompost addition rates during flowering and fruit setting are shown in Table 3. *Fv*, *Fm*, *Fv/Fm*, and *Fv/Fo* under BA_3, VA_3, BA_5, and VA_5 treatments, respectively, were significantly increased compared with those under CK treatment ($p < 0.05$). The highest *Fv*, *Fm*, *Fv/Fm*, and *Fv/Fo* were observed after VA_3 treatment, which increased by 17%, 12%, 4%, and 25%, respectively, compared with CK, where *Fv* and *Fv/Fo* of VA_3 were significantly higher than other treatments. On the contrary, BA_3 and BA_5 showed no statistically significant difference.

Table 3. Effect of BA and VA rates on chlorophyll fluorescence parameters.

Fluorescence Parameters	*Fo*	*Fv*	*Fm*	*Fv/Fm*	*Fv/Fo*
CK	3490 ± 45 ^{a}	$13{,}253 \pm 132$ ^{c}	$16{,}766 \pm 416$ ^{b}	0.791 ± 0.013 ^{b}	3.80 ± 0.03 ^{d}
BA_3	3300 ± 86 ^{bc}	$15{,}061 \pm 316$ ^{b}	$18{,}390 \pm 358$ ^{a}	0.819 ± 0.008 ^{a}	4.56 ± 0.09 ^{b}
VA_3	3278 ± 55 ^{c}	$15{,}523 \pm 203$ ^{a}	$18{,}816 \pm 268$ ^{a}	0.825 ± 0.002 ^{a}	4.74 ± 0.04 ^{a}
BA_5	3266 ± 42 ^{c}	$15{,}095 \pm 214$ ^{b}	$18{,}365 \pm 301$ ^{a}	0.822 ± 0.002 ^{a}	4.62 ± 0.03 ^{b}
VA_5	3380 ± 63 ^{b}	$15{,}080 \pm 185$ ^{b}	$18{,}496 \pm 240$ ^{a}	0.815 ± 0.001 ^{a}	4.46 ± 0.03 ^{c}

Note: BA for Biochar application and VA for Vermicompost application. ± indicates standard deviation. Means are not significantly different between different BA and VA rates when followed by the same lowercase letter; means are significantly different between BA and VA rates ($p \leq 0.05$) when followed by different lowercase letters. The same below.

As shown in Figure 3a, *RC/CSm, ABC/CSm*, *TRo/CSm*, and *ETo/CSm* were increased, and *DIo/CSm* was decreased by biochar and vermicompost application. BA_3 treatment had the highest *RC/CSm*, which was significantly higher than CK by 22%, and VA_3 and VA_5 showed no statistically significant difference. *ABC/CSm*, *TRo/CSm*, and *ETo/CSm* of VA_3 were significantly ($p < 0.05$) higher than other treatments (Figure 3b,c). The highest *ABC/CSm*, *TRo/CSm*, and *ETo/CSm* were observed in VA_3 treatment, which increased by 10%, 15%, and 30%, respectively, compared with CK treatment. Meanwhile, the increase in *ABC/CSm*, *TRo/CSm*, and *ETo/CSm* in BA_3, VA_3, BA_5, and VA_5 treatments were accompanied by a decrease in *DIo/CSm*, in which VA_3 treatment had the lowest *DIo/CSm*, with a decrease of 6% compared with CK, indicating that the application of biochar and vermicompost was effective in reducing the heat dissipation energy per cross section.

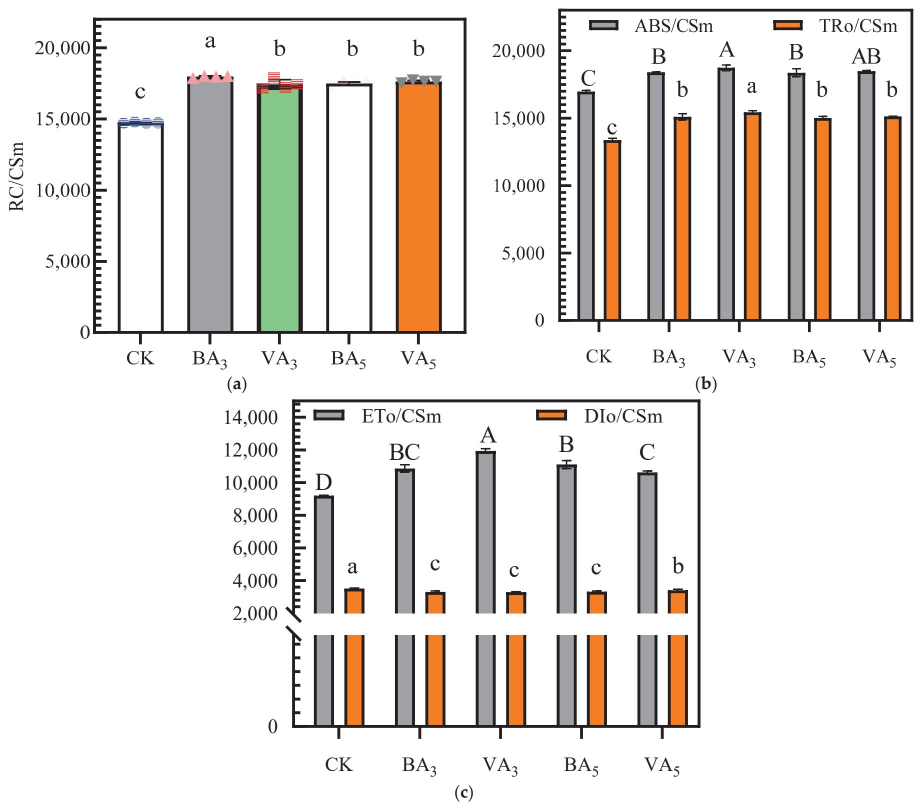


Figure 3. Effect of BA and VA rates on light energy absorption, capture, and transfer: (**a**) Effect of BA and VA rates on the *RC/CSm*. (**b**) Effect of BA and VA rates on the *ABC/CSm* and *TRo/CSm*. (**c**) Effect of BA and VA rates on the *ETo/CSm* and *DIo/CSm*. Note: BA represents biochar application and VA vermicompost application. Data are presented as mean ± standard error (n = 4). The meanings of the circles, triangles, and rectangles on the bars in (**a**) are indicated as duplicate data points for the different treatments. Means of *RC/CSm*, *TRo/CSm*, and *DIo/CSm* are significantly different between BA and VA rates ($p \leq 0.05$) when followed by different lowercase letters. Means of *ABC/CSm* and *ETo/CSm* are significantly different between BA and VA rates ($p \leq 0.05$) when followed by different uppercase letters. *RC/CSm* indicates the number of active reaction centers per CS; *ABS/CSm* indicates the absorption flux per CS; *TRo/CSm* indicates the trapped energy flux per CS; *ETo/CSm* indicates the electron transport flux per CS; *DIo/CSm* indicates the non-photochemical quenching per CS.

3.3. Yield and WUE of Tomato

Biochar and vermicompost application rates significantly ($p < 0.05$) influenced the tomatoes' average yield and *WUE* (Table 4). The yield parameters significantly increased with the application of BA and VA rates, whereas the average single-fruit weight of the first inflorescence and second inflorescence in BA_3, VA_3, BA_5, and VA_5 was not statistically significant. In addition, the first inflorescence of each treatment was higher than that of the second inflorescence. When the *TWU* of each treatment was 1425 $m^3 \cdot hm^{-2}$, the WUE was linearly correlated with yield; the highest yield and *WUE* (59.0 $t \cdot hm^{-2}$ and 41.4 $kg \cdot m^{-3}$, respectively) were observed in the VA_3 treatment, and *Y* and *WUE* under BA_3, VA_3, BA_5, and VA_5 treatments were 21%, 33%, 23%, and 25% higher, respectively, than those under CK treatment. The results indicated that the vermicompost had a better effect on increasing yield and *WUE* than biochar with the same application rates.

Table 4. Effects of BA and VA rates on tomato yield and water use efficiency.

Treatments	Average Fruit Weight Per Fruit (g)		Yield Per Plant (g)	Yield ($t \cdot hm^{-2}$)	*WUE* ($kg \cdot m^{-3}$)
	First Inflorescence	Second Inflorescence			
CK	135 ± 17^{b}	112 ± 4^{b}	990 ± 76^{c}	44.5 ± 3.4^{c}	31.2 ± 2.4^{c}
BA_3	150 ± 14^{ab}	149 ± 4^{a}	1196 ± 68^{b}	53.8 ± 3.1^{b}	37.8 ± 2.1^{b}
VA_3	173 ± 12^{a}	155 ± 18^{a}	1312 ± 63^{a}	59.0 ± 2.8^{a}	41.4 ± 2.0^{a}
BA_5	157 ± 23^{ab}	148 ± 8^{a}	1220 ± 77^{ab}	54.9 ± 3.5^{ab}	38.5 ± 2.4^{ab}
VA_5	169 ± 14^{a}	141 ± 7^{a}	1240 ± 48^{ab}	55.8 ± 2.2^{ab}	39.2 ± 1.5^{ab}

Note: BA for Biochar application and VA for Vermicompost application. ± indicates standard deviation. Means are not significantly different between different BA and VA rates when followed by the same lowercase letter; means are significantly different between BA and VA rates ($p \leq 0.05$) when followed by different lowercase letters. The same below.

3.4. Correlation Analysis of Plant Physiological Indicators and Tomato Yield

Pearson correlation analysis results among photosynthetic parameters (*Pn*, *Ci*, *Tr*, and *Gs*), chlorophyll fluorescence traits (*Fv/Fm*, *Fv/Fo*, *ABC/CSm*, *TRo/CSm*, *ETo/CSm*, and *DIo/CSm*), and yield are displayed in Table 5. *Pn*, *Ci*, *Tr*, *Fv/Fm*, *Fv/Fo*, *ABC/CSm*, *TRo/CSm*, *ETo/CSm*, and yield had a strong positive correlation (*R* > *0.6*), apart from *Gs*, with *ETo/CSm* and yield. These factors showed a strong negative correlation with *DIo/CSm*. In addition, photosynthetic parameters (*Pn*, *Ci*, and *Tr*) showed a strongly significant correlation with chlorophyll fluorescence traits (*Fv/Fm*, *Fv/Fo*, *ABC/CSm*, *TRo/CSm, ETo/CSm*, and *DIo/CSm*). Moreover, *Pn*, *Ci*, *Fv/Fo*, *ABC/CSm*, *TRo/CSm*, and *ETo/CSm* showed a highly significant correlation (*R* > *0.8*) with yield, indicating that the increment in net photosynthesis and light energy absorbed, trapped, and transported per cross section of plant leaves could increase tomato yield.

Table 5. Correlation analysis between plant physiological indicators and tomato yield.

Indices	*Pn*	*Ci*	*Tr*	*Gs*	*Fv/Fm*	*Fv/Fo*	*ABS/CSm*	*TRo/CSm*	*ETo/CSm*	*DIo/CSm*	*Yield*
Pn	1										
Ci	0.960	1									
Tr	0.918	0.936	1								
Gs	0.660	0.759	0.696	1							
Fv/Fm	0.899	0.895	0.869	0.626	1						
Fv/Fo	0.939	0.975	0.932	0.663	0.911	1					
ABS/CSm	0.950	0.947	0.903	0.635	0.829	0.949	1				
TRo/CSm	0.962	0.964	0.925	0.661	0.857	0.961	0.969	1			
ETo/CSm	0.904	0.905	0.886	0.463	0.854	0.943	0.883	0.900	1		
DIo/CSm	−0.775	−0.816	−0.871	−0.608	−0.795	−0.860	−0.729	−0.774	−0.859	1	
Yield	0.876	0.826	0.775	0.485	0.725	0.821	0.843	0.817	0.800	−0.686	1

Note: Pearson correlation coefficient ranging from 0.8 to 1.0 indicates very strong correlation, from 0.6 to 0.8 indicates strong correlation, from 0.4 to 0.6 indicates medium correlation, from 0.2 to 0.4 indicates weak correlation, and from 0.0 to 0.2 indicates very weak correlation or no correlation. "-" represents a negative correlation.

4. Discussion

4.1. BA and VA Rates Improved Photosynthesis and Chlorophyll Fluorescence Traits

Biochar and vermicompost rates had a positive effect on photosynthetic parameters and chlorophyll fluorescence traits of treated plants. VA_3 treatment had the highest *Pn*, *Ci*, *Fv*, *Fm*, *Fv/Fm*, *Fv/Fo*, *ABC/CSm*, *TRo/CSm*, and *ETo/CSm*, which increased by 49%, 65%, 17%, 12%, 4%, 25%, 10%, 15%, and 30%, respectively, compared with CK. Photosynthesis is the basis for crop yield and quality formation, and 95% of organic matter in crops is derived from photosynthesis [42]. Previous studies illustrated the improvement of water- and fertilizer-holding capacity of soil, enhanced photosynthesis of plant leaves, and increased *Pn*, *Tr*, and *Gs* by biochar [43,44]. Our results are supported by Cui et al. [45], who revealed that biochar significantly improved the photosynthesis of plants, and *Pn*, *Tr*, and *Gs* of 3% biochar-treated plants increased by 94%, 35%, and 35%, respectively, compared with the control. As described by Shi et al. [46], the application of vermicompost increased *Tr*, *Gs*, *Pn*, and *Ci* of tomato plant leaves by 84%, 52%, 21%, and 43%, respectively. Chlorophyll fluorescence parameters are closely related to various reaction processes in photosynthesis [47]. The energy changes in photosynthesis can be reflected by chlorophyll fluorescence-induced kinetic curves. Chlorophyll fluorescence can sensitively reflect changes in leaf photosynthesis and is a probe for studying photosynthesis [48–50]. Zhang et al. [51] reported that biochar reduced the shutdown of active reaction centers in alfalfa leaves, increased *Fv/Fm* of *PSII*, and enhanced photosynthesis. Fan et al. [52] showed that biochar increased *Fo* and *Fv/Fm* of plants, enhanced photosynthetic performance, and promoted plant growth and development. Yang et al. [53] found that the relative chlorophyll content of winter wheat was highly significantly and positively correlated with *RC/CSm* and *ETo/CSm*, and that *RC/CSm* and *ETo/CSm* were all related to the photosynthetic efficiency of plants, while the level of chlorophyll content reflected the strength of photosynthetic efficiency of plants and changes in fluorescence parameters [54]. Our results are also consistent with the results of Wang et al. [55], who reported that spraying exogenous phytohormones alleviated the impairment of light energy use and overall photosystem II performance in sweet potato leaves by drought stress, with good linear relationships for *Pn*, *Gs*, *Fv/Fm*, *ETo/CSm* and *ABS/CSm*. In our study, the effect of vermicompost in improving photosynthesis and chlorophyll fluorescence was more effective than that of biochar at the same application rate, as indicated by the *N* and *P* contents of the experimental vermicompost, which were 1.45 and 8.17 times higher than those of biochar (Table 1), thereby enhancing soil fertility and promoting the growth and development of tomato plants. This finding was consistent with the conclusion of previous studies, that is, vermicompost was richer in nutrients than biochar, which could remarkably enhance soil fertility [56,57].

4.2. Yield and WUE in Response to BA and VA Rates

The experimental results revealed significant enhancements in the yield parameters under BA- and VA-amended treatments. The application of biochar and vermicompost significantly increased the average single-fruit weight of tomatoes compared with CK (Table 4). Consequently, the total yield of each treatment was also significantly increased. Under the present experimental conditions, irrigation levels were consistent among the treatments; therefore, the BA and VA rates significantly improved *WUE*. The results are consistent with those of Blouin et al. [58], who revealed that the application of VA rates significantly increased the yield and biomass of crops. Wang et al. [59] also showed that the VA-amended treatment significantly increased tomato yield. These results were consistent with those of Zhang et al. [60] and Akhtar et al. [61], who reported that the application of biochar promoted the ability of tomato plants to absorb nutrients and increased tomato yield and *WUE*. This study also found that the application of vermicompost was more effective in improving tomato yield than biochar at the same application rate probably because the application of vermicompost under the experimental conditions increased the effectiveness of the nutrients required by the crop and promoted the photosynthesis and growth of the plant, thereby significantly increasing yield and *WUE* [62]. Our results are also supported by Ding et al. [63], who revealed that vermicompost and biochar significantly increased the yield of tomatoes every year compared with the control, and the biomass accumulation showed that vermicompost was better than biochar. Moreover, in our study, the application of 3% vermicompost (equivalent to 16,200 kg·hm^{-2}) increased tomato yield by up to 32.56%, which was better than the 5% vermicompost treatment, probably because the excessive accumulation of humic acid in the high content (5%) of vermicompost inhibited plant growth and development, resulting in lower yield [64,65]. As described by Wu et al. [66], among the treatments applying different rates of vermicompost (7500, 15,000, and 22,500 kg·hm^{-2}), the highest tomato yield was obtained in the 15,000 kg·hm^{-2} treatment, which was consistent with the results of our study.

5. Conclusions

The results showed that BA- and VA-amended treatments had a positive effect on improving photosynthetic parameters and chlorophyll fluorescence traits, particularly at the 3% application rate of vermicompost. The application of biochar and vermicompost effectively increased photosynthetic parameters (*Pn*, *Ci*, and *Tr*) as shown by studying the improvement of chlorophyll fluorescence traits (*Fv/Fm*, *Fv/Fo*, *ABC/CSm*, *TRo/CSm*, *ETo/CSm*, and *DIo/CSm*), producing organic substances needed by the crop, thereby increasing yield. Moreover, vermicompost with the same rate was significantly more effective in enhancing *Pn*, *Ci*, *Fv*, *Fm*, *Fv/Fm*, *Fv/Fo*, *ABC/CSm*, *TRo/CSm*, and *ETo/CSm* of plants than biochar, resulting in significantly higher yield in VA_3 and VA_5 than in BA_3 and BA_5. The *WUE* of plants under CK, BA_3, VA_3, BA_5, and VA_5 treatments increased from 31.2 to 37.8, 41.4, 38.5, and 39.2 kg·m^{-3}, respectively. The results of Pearson correlation analysis revealed that biochar and vermicompost rates improved the net photosynthesis and light energy absorbed, trapped, and transported per cross section of plant leaves, which could increase tomato yield. These results indicated that vermicompost was more effective than biochar at the same rate in improving photosynthesis as shown by studying chlorophyll fluorescence and increasing tomato yield, particularly at 3% application rate.

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Additive and Non-Additive Effects on the Control of Key Agronomic Traits in Popcorn Lines under Contrasting Phosphorus Conditions

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Abstract: Phosphorus is a non-renewable natural resource that will run out of reserves in the upcoming decades, making it essential to understanding the inheritance of nutrient use efficiency for selecting superior genotypes. This study investigated the additive and non-additive effects of commercially relevant traits for the popcorn crop (grain yield—GY, popping expansion—PE, and expanded popcorn volume per hectare—PV) in different conditions of phosphorus (P) availability in two locations in Rio de Janeiro State, Brazil. Six S_7 lines previously selected for P use—L59, L70, and P7, efficient and responsive; and L54, L75, and L80, inefficient and non-responsive—were used as testers in crosses with 15 progenies from the fifth cycle of intrapopulation recurrent selection of UENF-14, with adaptation to the North and Northwest regions of Rio de Janeiro State. Using the Griffing diallel analysis, P use efficiency was predominantly additive in the expression of PE, and non-additive effects were prominent for GY and PV. For obtaining genotypes that are efficient for phosphorus use, it is recommended that heterosis with parents that provide additive gene accumulation for PE be explored.

Keywords: abiotic stress; genetic control; Griffing diallel analysis; *Zea mays* var. *everta*

1. Introduction

Phosphorus (P) is the second most consumed nutrient in agriculture, surpassed only by nitrogen, and is limiting for agricultural productivity worldwide [1]. Despite playing a crucial role in crop productivity, its soil reserves (organic and inorganic forms) have limited supply to the plants because of its fixation and formation of complexes with other soil nutrients [2,3].

Maize (*Zea mays*) is one of the most widely cultivated species of commercial interest, both as a staple food and for industrial use, in tropical and temperate climatic soils in the world. Under cultivation, especially in acidic and alkaline soils, large amounts of P fertilizer are applied to maize fields to maximize yields. The consequence is high amounts of high-cost phosphate fertilizers being applied to P-deficient soils to achieve maximum yields to guarantee food security for the growing world population, which will hit 10 billion inhabitants by 2050 [4]. Therefore, to meet this demand, the global production of phosphate fertilizers will require a significant increase in phosphate extraction in the next decades. This is, however, a non-renewable resource that is likely to become scarcer as its use becomes more frequent [5].

Accordingly, promoting phosphorus use efficiency in crop plants is key to sustainable agricultural development; this is especially important in tropical and subtropical regions, where there is greater loss of the nutrient due to high temperatures and heavy rainfall, together with the fixation of the nutrient by iron and aluminum oxides in the soil, resulting in the loss of about 70–80% of the nutrient applied to crops [6–8].

Phosphorus deficiency in soil leads to physiological and biochemical disturbances in plants. As a result, they show a reduction in leaf area, height, dry matter, and major metabolic activities [9]. This is because phosphorus plays a fundamental part in the production of energy and enzyme activation, in addition to being a structural element of nucleic acids and phospholipids and participating in processes such as cell division [8]. P deficiency generates significant impacts on commercially important crops, among them popcorn (*Zea mays everta*), causing large losses in yields.

The knowledge of the genetic basis regarding agronomic traits of interest is extremely relevant for plant breeding programs that seek to increase crop yield in areas where there is low soil-nutrient availability by means of generating superior hybrids and segregants for limiting conditions [10]. To this end, diallel crosses are widely used in cultivated species to provide genetic information by estimating the combining abilities of the parents and hybrids [11]. This strategy enables the estimation of the existence of additive effects from the parents and non-additive effects in crosses [12]. Diallel analysis, however, may become impractical depending on the number of lines to be used, requiring large experimental areas and labor in manual crosses. To solve this problem, the testcross method proposed by Davis [13] has been used as an option, allowing the evaluation of many lines in crosses with testers. By doing so, lines with inferior agronomic performance may be eliminated. As a result, the crosses show the most promising lines, thus making the procedure more effective [14].

Previous studies carried out for popcorn have proven the genetic action of the main traits of economic importance in the crop. In environments with adequate water and nutrient supply [15–17] and under water-deficit conditions [18,19] as well as P and N stress [10,20,21], additive genetic action prevails for grain popping expansion, while non-additive action has been the most important in the expression of grain yield under water stress [14,18,19]. These results are also consistent with studies conducted with biotic stressors [22–26].

Despite the relevance, only one research paper has been developed so far [10] toward understanding the influence of additive and non-additive effects on popcorn, a crop that earns about USD 1 billion annually in the United States. Given this, this study was considered relevant to contributing to filling the current gaps in knowledge regarding the genetic control of economically important traits for popcorn under phosphorus-limiting conditions. Therefore, the general and specific combining abilities and the genetic merit of 90 testcross hybrids of popcorn were estimated, and inferences were made about their additive and non-additive gene effects on the key agronomic traits of the crop. The goal was to propose breeding strategies for popcorn to develop cultivars adapted to phosphorus-limiting conditions as an option for leveraging Brazilian agribusiness.

2. Results

2.1. Genetic Variability for Agronomic Traits Evaluated under Contrasting Phosphorus Conditions in Both Environments

A significant difference between the genotypes (G) at a 1% probability level ($p \leq 0.01$) was detected using the F test for all the traits (grain yield—GY, popping expansion—PE, and expanded popcorn volume—PV) for both levels of phosphorus availability, in Campos dos Goytacazes and Itaocara. The source of variation in phosphorus availability (P) and the G $\times$ P interaction also exhibited significant effects ($p \leq 0.01$) for all the traits evaluated in the two locations studied (Table 1).

Table 1. Summary of the analysis of variance, of the general combining ability (GCA), and of the specific combining ability (SCA) for the agronomic traits evaluated in testcrosses under contrasting conditions of phosphorus in soil in Campos dos Goytacazes and Itaocara.

SV	DF	Mean Squares					
		Campos dos Goytacazes			Itaocara		
		GY	PE	PV	GY	PE	PV
Block/Phosphorus	4	554,624.5	9.5	529.0	103,022.6	3.3	116.5
Genotype (G)	89	954,080.6 **	69.6 **	1101.1 **	992,731.8 **	87.40 **	1414.1 **
GCA I	14	1,672,189.3 **	247.9 **	2585.7 **	2,244,297.7 *	314.3 **	4149.6 **
GCA II	5	4,395,517.2 *	279.9 **	2618.4 ^{ns}	4,047,292.2 *	398.7 **	5616.3 *
SCA	70	564,640.9 **	18.9 **	695.8 **	524,233.5 *	19.8 ^{ns}	566.9 ^{ns}
Phosphorus (P)	1	36,456,840.8 **	246.3 **	37,105.2 **	17,515,196.9 **	255.7 **	27,642.8 **
G × P	89	330,925.0 **	14.6 **	317.0 **	423,063.8 **	16.7 **	509.3 **
GCA I × P	14	439,193.5 **	33.0 **	287.6 ^{ns}	423,063.9 **	13.9 **	848.3 **
GCA II × P	5	684,529.0 **	20.6 *	956.3 **	845,321.8 **	8.8 **	868.8 **
SCA × P	70	284,013.2 **	10.5 **	277.2 **	727,343.1 **	17.8 **	415.9 **
Residue	356	178,327.5	7.0	179.3	209,610.6	2.8	171.1
Mean		2514.8	27.6	69.6	3143.7	26.2	82.4
CVe (%)		16.8	9.6	19.2	14.6	6.4	15.9

** and *—significant at 1% and 5% level probability using the F test; and ^{ns}—not significant at 5% probability using the F test; SV—source of variation; DF—degree of freedom; CVe (%)—experimental coefficient of variation; PE—popping expansion; GY—grain yield; and PV—expanded popcorn volume per hectare.

Regarding the effects of the general combining ability of the progenies (GCA I) and the testers (GCA II), the estimates were significant for all traits except for PV in Campos dos Goytacazes. Analyzing the mean square values for specific combining ability (SCA), it was observed that, for the GY, PE, and PV traits, there were highly significant values ($p \leq 0.01$) in Campos dos Goytacazes. In Itaocara, there was only significance for GY at a 5% probability level (Table 1).

There was high significance for the GY and PE traits in Campos dos Goytacazes and for GY, PE, and PV in Itaocara regarding the progeny interaction with phosphorus availability (GCA I × P). The tester interactions with phosphorus levels (GCA II × A) and between specific combining ability and phosphorus levels (SCA × P) were found to be significant for all the traits evaluated.

2.2. *General Combining Ability Effects*

General combining ability (GCA) estimates provide information about the additive effects of genes for the traits studied. From this, it may be stated that lines L682, L688, and L686, in Campos dos Goytacazes, showed the three highest values—338.431, 310.452, and 290.454, respectively—for GY in the environment without induced stress. In turn, for the low-phosphorus environment, the most positive values were assigned to the genotypes L688, L695, and L689 (313.311, 280.976, and 207.294, respectively) (Figure 1).

Considering the testers in question for GY, three—L70, L59, and L75—in the environment with high phosphorus levels and four—L80, L59, L70, and L75—in the environment with low phosphorus levels displayed positive deviations. Three of the best lines used as testers—L70, L59, and L75—presented positive values in both phosphorus conditions, and L59 was the tester that showed the lowest variation when comparing 367 to 739, ranking well in both high and low soil-phosphorus conditions in the Campos dos Goytacazes environment (Figure 1).

For the general combining ability effect for the PE trait, eight lines at the optimal phosphorus level and seven lines at the low phosphorus level showed positive values for PE, especially for L681, L689, and L690 at the optimal level and L681, L688, and L689 at the low P level (Figure 1). Lines L681 and L689 demonstrated the highest effects of GCA and the lowest variations when both environments were analyzed. Favorable performance was seen for the P7 and L54 lines in the environment with high phosphorus levels when evaluating the testers for PE. As for the phosphorus-deficient environment, the testers with positive values were P7, L80, and L54. Thus, the tester P7 achieved good performance regardless of the environmental conditions.

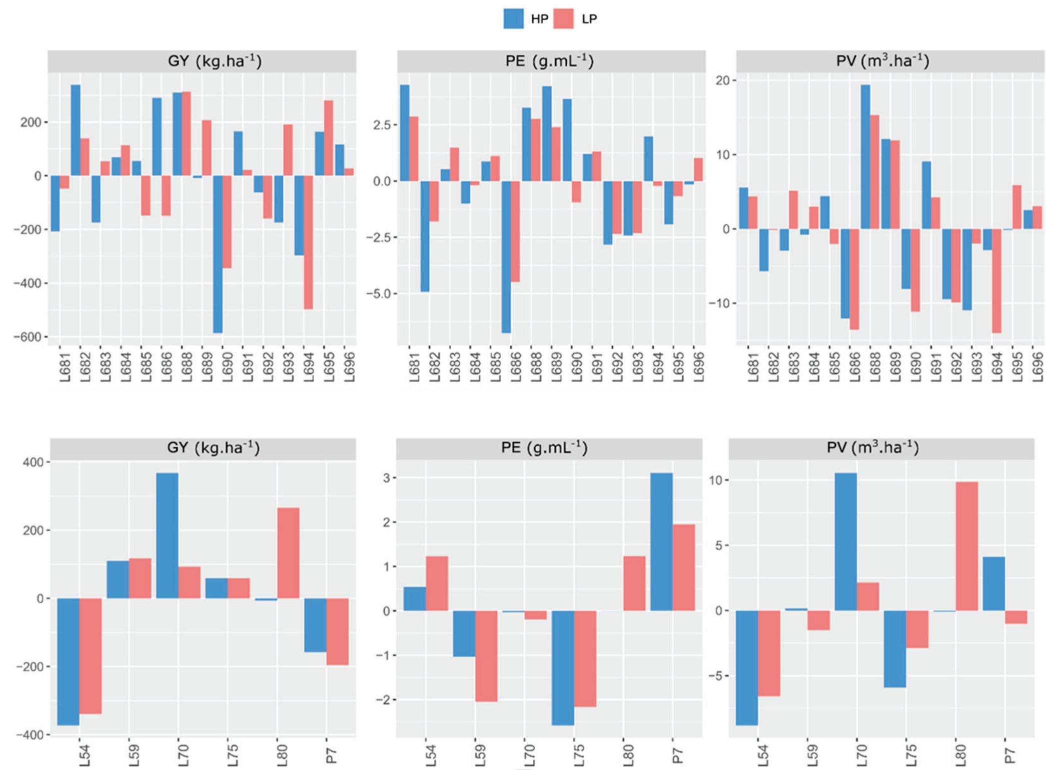

Figure 1. Estimates of general combining ability effects ($\hat{g}i$) for three traits evaluated in 21 popcorn parents in a partial diallel scheme in Campos dos Goytacazes. HP—high phosphorus level; LP—low phosphorus level; PE—popping expansion; GY—grain yield; and PV—popcorn expanded volume per hectare; (−) signal indicating negative values.

For the PV trait, the GCA estimates identified six lines—L688, L689, L691, L681, L685, and L696—with positive values in the environment with optimal phosphorus availability. On the other hand, in the environment with a low supply of the nutrient for this same trait, eight lines—L688, L689, L695, L683, L681, L691, L696, and L684—showed positive values. In the simultaneous analysis of the environments, the lines with the best results were L688 and L689, respectively. Additionally, considering the PV, of the six testers, three—L70, P7, and L59—expressed positive results in the environment with high phosphorus levels. In the environment with stress, only two testers—L80 and L70—had positive values. Among the genotypes with good results, tester line L70, from the BRS Angela population, showed positive values for PV at both phosphorus levels in Campos dos Goytacazes (Figure 1).

The general combining ability effects for GY in Itaocara indicated that lines L694, L689, L682, L684, L686, L688, L690, and L683 had positive deviations from the mean in the environment with fertilizer recommendations. Under the nutrient-deficiency restriction, the best lines were L689, L694, L685, L688, L693, L682, L684, and L695. Given the best performances, lines L694, L689, L682, L684, and L688 were in both study environments. Thus, it should be stressed that lines L694 and L689 had the best positions in the ranking (Figure 2).

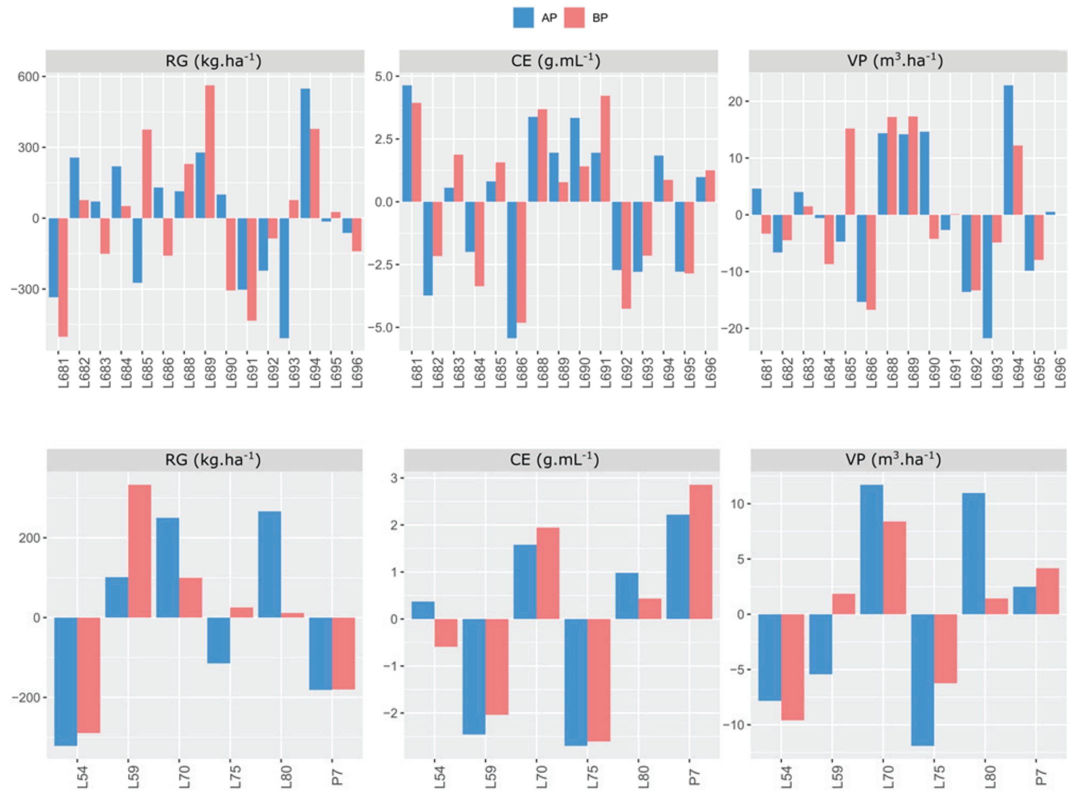

Figure 2. Estimates of general combining ability effects ($\hat{g}i$) for three traits evaluated in 21 popcorn parents in a partial diallel scheme in Itaocara. HP—high phosphorus level; LP—low phosphorus level; PE—popping expansion; GY—grain yield; and PV—popcorn expanded volume per hectare; (−) signal indicating negative values.

As for the $\hat{g}i$ effects of the testers for GY in Itaocara, three lines—L80, L70, and L59—showed positive values in the environment with satisfactory fertilization, while in the environment with induced stress, four genotypes—L59, L70, L75, and L80—were seen as the most relevant. It should be observed that, when analyzing the ranking of the two environments, tester L70 remained stable, ranking second, surpassed only by lines L80 and L59 in the environment with fertilizer recommendation and phosphorus restriction, respectively (Figure 2).

The evaluations conducted in Itaocara showed the following hierarchical ranking of the lines with positive values for the PE trait in the environment where phosphorus was applied: L681, L688, L690, L689, L691, L694, L696, L685, and L683. For low levels of phosphorus in the soil, however, there were changes in the performances of the lines, which began to be arranged according to the ranking of best performances, as follows: L691, L681, L688, L683, L685, L690, L696, L694, and L689 (Figure 2). Regardless of the interaction with the phosphorus levels, the genotypes expressing positive performance were the same. It is worth noting, however, that changes were caused in the ranking of the best genotypes, and that lines L681 and L688 displayed good stability, maintaining high positive values. Regarding the effect of the six testers studied, it may be stated that there was no change resulting from the environments, especially for P7, L70, and L80, in the expression of positive values (Figure 2).

The evaluations performed in Itaocara indicated that, for the trait of popcorn expanded volume per hectare (PV), lines L694, L690, L688, L688, L689, L681, L683, and L696; and L689, L688, L685, L694, L683, L691, and L696, were the most prominent in environments with optimal and low phosphorus levels, respectively. Among these, lines L694, L688, and L689 expressed positive values higher than 12.201 in a range from −21.73 to 22.80. This confirms the good performance of these lines when considering the effects of the general combining ability of the S_7 progenies (Figure 2). The PV trait also allowed for discrimination of the testers L70, L80, and P7 in the environment with a phosphorus supply. As for the nutrient-deficient environment, the genotypes that expressed the highest positive values were: L70, P7, L59, and L80. Tester L70 showed good performance in both environments, with positive values of 11.713 and 8.391, respectively, when evaluated in Itaocara (Figure 2).

Considering the set of traits evaluated for $\hat{g}i$, the lines with the best performance for GY and PE in the environment with high phosphorus availability in Campos dos Goytacazes were: L682, L688, and L686; and L681, L689, and L688, respectively. For the PV variable, the genotypes with the highest positive values were: L688, L689, L691, and L681. Of special relevance is the phenotypic plasticity of line L688, which exhibited positive values for all the variables, suggesting that there is a range of favorable alleles in this parent.

The best lines for GY in the stressed environment were L688, L695, and L689, and for PE, L681, L688, and L689. It is emphasized that lines L688, L689, and L695 ranked highest for PV. Particularly noteworthy is the good positive deviation performance of L688 and L689 in the nutrient-deficient environment, confirming the presence of favorable alleles for phosphorus use efficiency in the parents from the UENF-14 population (Figures 1 and 2).

When the set of testers was evaluated, it can be seen that, for GY and PE, the genotypes L70 and L59, and P7 and L54, respectively, had good performances in the environment with the optimal phosphorus level in Campos dos Goytacazes. Regarding the PV trait, the most prominent genotypes were L70, P7, and L59, as they showed the highest positive values. In the environment with low phosphorus levels, a change in the performance of the testers was observed, in which the highest positive values were seen for lines L80, L59, and L70 relative to the GY trait. The most relevant genotypes for PE were P7, L80, and L54. The PV allowed tester lines L80 and L70 to stand out in presenting the highest positive values.

When comparing the individual values of GY and PE for the PV trait in relation to the experiments carried out in Itaocara, the genotypes L694, L690, and L688 gave the best performances. The order of the most positive values for variables GY and PE was attributed to lines: L694, L689, L682; and L681, L688, L690, respectively. Based on the considerations, line L688 showed good performance for both PV and PE and is considered a promising genotype. Regarding the environment with low phosphorus levels, the lines with the best performances were L689, L694, and L685 for GY; and L691, L681, and L688 for PE. Considering the PV, the genotypes with the highest positive values were L689, L688, and L685 (Figures 1 and 2).

Upon analyzing the testers when grown in Itaocara, differences in performance could also be identified. The most prominent positive values were found in L80, L70, and L59 for GY; and in P7, L70, and L80 for PE in the environment with the proper adjustments for phosphorus. Considerations carried out for PV allowed us to indicate the parents L70, L80, and P7 as the most promising. For the nutrient-deficient condition, the variable PV enabled us to distinguish lines L70, P7, and L59 as having the most positive values. For GY and PE, the genotypes L59, L70, and L75; and P7, L70, and L80, respectively, were the most relevant in this same environment.

2.3. Genetic Merit of Hybrids

Specific combining ability effects (SCA—$\hat{s}_{ij}$) are the result of the presence of dominance gene effects. The effects of $\hat{s}_{ij}$ for GY in Campos dos Goytacazes showed 46 hybrids with positive variances, suggesting that the dominance gene effects prevail, which expressed amplitudes from 2.289 to 616.921 in the environment with phosphorus supplementation. The three hybrid combinations that exhibited the highest heterosis, considering $\hat{s}_{ij}$ for this trait, were L691 × P7, L686 × L80, and L689 × L59. Among the testers, the parents L75, L54, and L80 were involved in most crosses with positive SCA values, with magnitudes of 21.73%, 17.39%, and 17.39%, respectively (Figure 3).

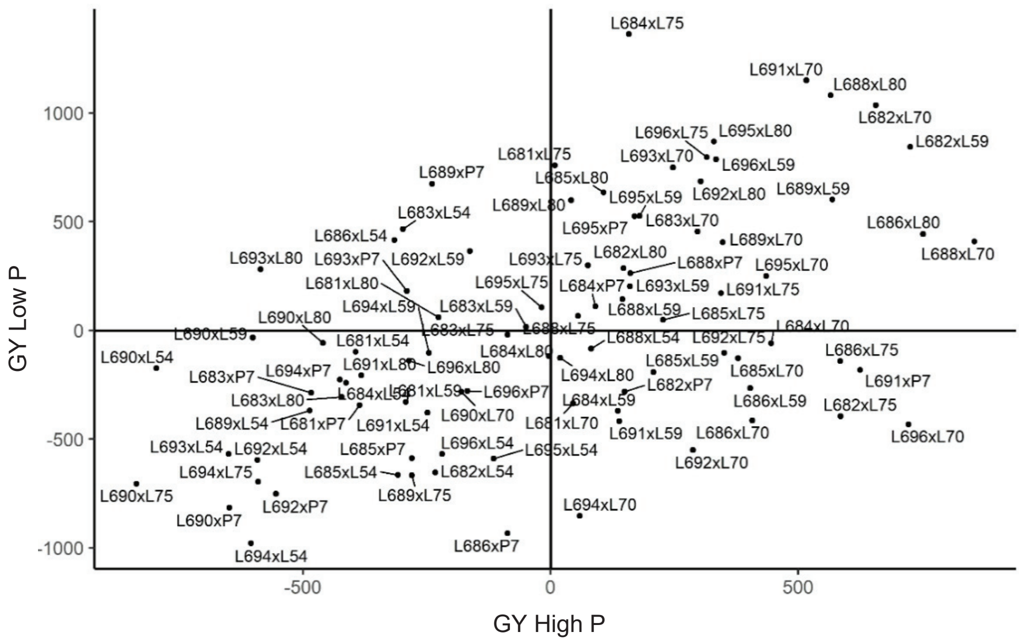

Figure 3. Estimates of the genotypic value ($\hat{s}_{ij}$) of hybrids for grain yield (GY) in diallel hybrids of popcorn evaluated under high and low phosphorus availability in Campos dos Goytacazes. (−) signal indicating negative values.

On the other hand, in the environment with induced stress, the number of genotypes with positive values for $\hat{s}_{ij}$ was significantly reduced. Among the 90 hybrids tested, only 37 crosses expressed positive magnitudes, which ranged from 2.601 to 1189.951. The most vigorous genotypes for $\hat{s}_{ij}$ were L684 × L75, L691 × L70, and L686 × L54. As for the highest percentages of crosses with positive estimates in this case, they occurred with the testers L80 and L59, with values of 21.62% and 18.91%, respectively (Figure 3).

Therefore, the selection of parents based on additive genetic merit in Campos dos Goytacazes enabled the identification of genotypes with genetic potential to be phenotypically superior for each environment. In the experiment with fertilizer adjustments, the highest genetic merits were expressed in the combinations L88 × L70, L682 × L59, and L696 × L70, whereas in the nutrient-deficient environment, the hybrids L684 × L75, L691 × L70, and L688 × L70 expressed the highest genetic merits, exhibiting good allelic complementation (Figure 3).

The experiments conducted in Itaocara showed that the effects of $\hat{s}_{ij}$ enabled 49 hybrids to be discriminated, with heterosis values estimated from 22.423 to 631.804 in the environment with fertilization recommended for the popcorn crop. This selection indicated the crosses with the highest dominance gene effects, with the hybrids L691 × L59, L685 × P7, and L692 × L70 standing out as having the best performances. Regarding the estimates of the general combining ability, the testers L80, L70 and L59 showed positive values, being in the formation of the best genotypes. Among them, line L59 was the most frequent, present in 20% of the best hybrid combinations (Figure 4).

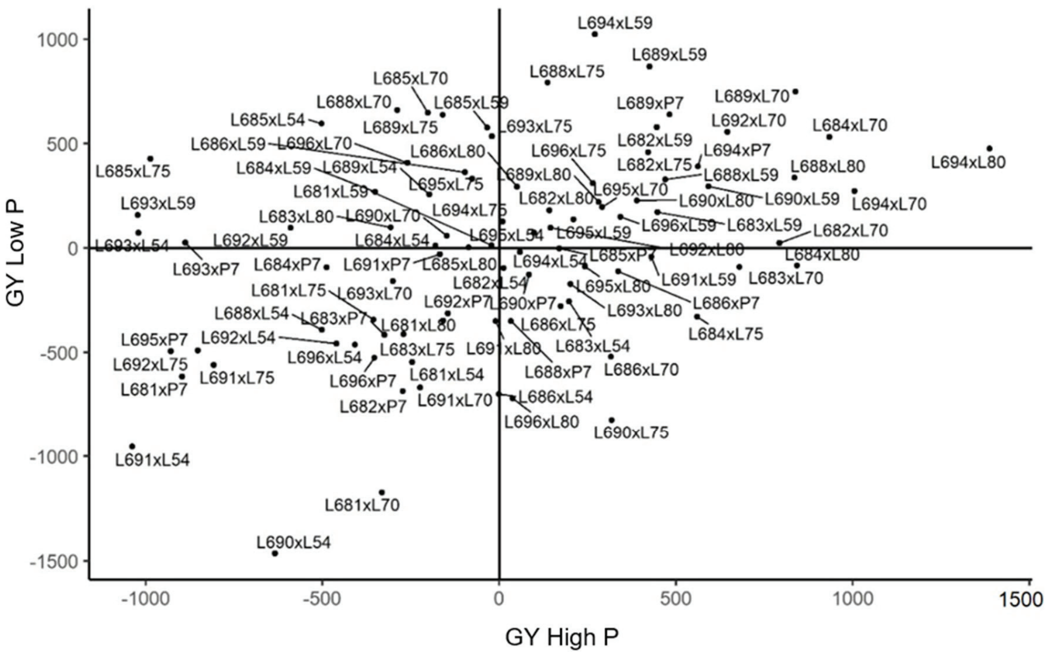

Figure 4. Estimates of the genotypic value ($\hat{s}_{ij}$) for grain yield (GY) in diallel hybrids of popcorn evaluated under high and low phosphorus availability in Itaocara. (−) signal indicating negative values.

In the experiments for GY carried out in Itaocara, there was no considerable reduction in the number of genotypes obtained in the environment with nutrient reduction. Thus, from 90 combinations, 51.10% of the crosses had positive values. Among them, three hybrids (L691 × P7, L692 × L70, and L688 × L75) stood out with high estimates of $\hat{s}_{ij}$, and the greatest dominance gene effect was shown by the combination L691 × P7, representing high allelic complementation in the parents. The most frequent tester in the crosses was line L80, as it participated in nine positive crosses, accounting for approximately 20% of the positive combinations in Itaocara. Analyzing the $\hat{g}i$ estimates of this tester, the additive effect of the genes is evident, since it showed positive estimates and high magnitudes in the nutrient-deficient environment (Figure 4).

From the estimates of additive genetic merit, it was observed, in the experiments conducted in Itaocara, that the values of GY emphasized the hybrids L694 × L59, L689 × L59, and L88 × L75 as having the highest estimates of heterosis when grown in an environment with nutrient stress. In the environments with added phosphorus, the best allelic complementation was found in L694 × L80 and L694 × L70 (Figure 4).

In relation to the PE variable, the evaluations conducted in Campos dos Goytacazes under conditions with phosphorus supplementation made it possible to point out the testers L59 and L70 due to their positive SCA values and the highest frequencies in the formation of hybrids with positive estimates of $\hat{s}_{ij}$, corresponding to 20% of the crosses. These parents, however, showed no additive gene effects for their $\hat{g}_i$ estimates. Additionally, for PE, 50% of the hybrid combinations showed positive magnitudes of $\hat{s}_{ij}$. However, the values showed a low range, varying from 0.166 to 3.534. The hybrids that exhibited the highest heterosis for SCA were L684 × L75, L691 × L75, and L684 × L54, respectively (Figure 5).

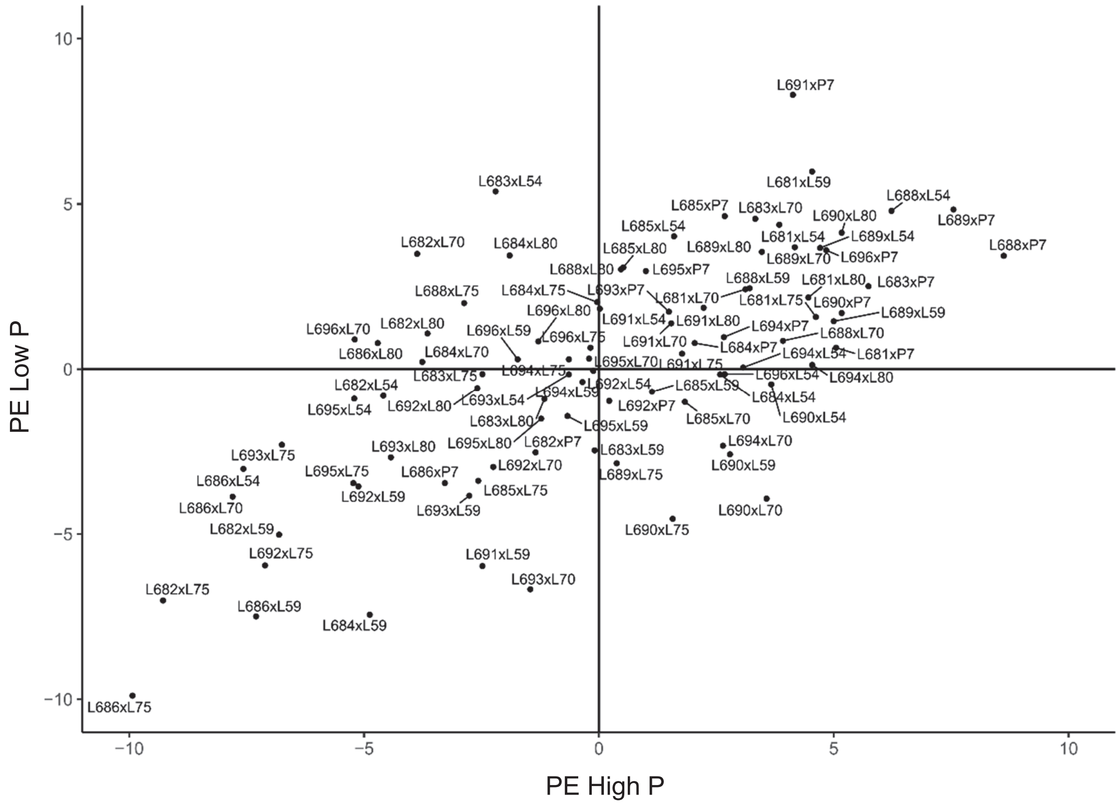

Figure 5. Estimates of the genotypic value ($\hat{s}_{ij}$) for popping expansion (PE) in diallel hybrids of popcorn evaluated under high and low phosphorus availability in Campos dos Goytacazes. (−) signal indicating negative values.

In the low-phosphorus environment, it was possible not only to obtain 47 hybrids with high gene complementation, in which the SCA estimates ranged from 0.053 to 5.483, but also to detect that the crosses L682 × L70, L681 × L59, and L691 × P7 had the highest SCA values. In terms of tester performance, line L59 had the greatest participation in the formation of superior hybrids, being the source of 21.27% of the best combinations (Figure 5).

When the genetic merit of the hybrids for PE was evaluated in both growing conditions in Campos dos Goytacazes, it was found that the hybrid combinations with the highest genetic merit were L691 × P7, L683 × L54, and L681 × L59 in a low-phosphorus environment, and L688 × P7, L689 × P7, and L688 × L54 in an environment with phosphorus supplementation (Figure 5).

In Itaocara, the experiments for PE inferred that the tester P7 had the highest percentages of allelic complementation in the crosses, with high estimates of $\hat{s}_{ij}$ for 22% of the hybrid combinations in the phosphorus supply condition, and 20.90% in the nutrient-deficient environment. Thus, based on the combining abilities, it can be stated that the tester P7 has genes favorable for phosphorus use efficiency, and also expresses more pronounced genetic divergence than the other parents (Figure 6).

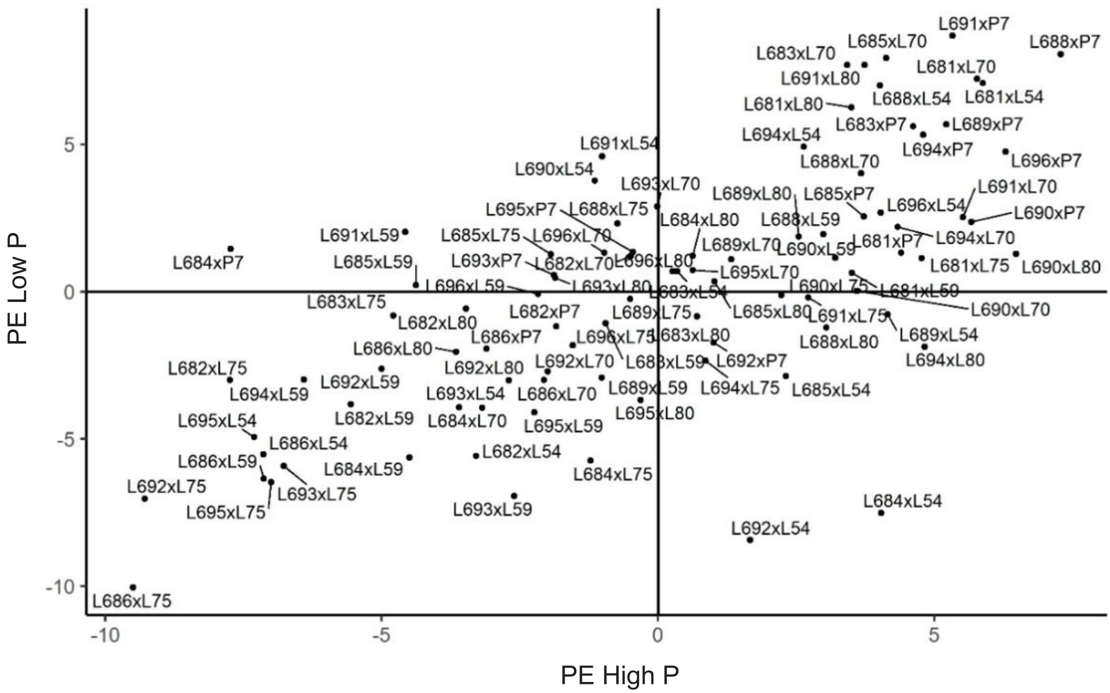

Figure 6. Estimates of the genotypic value ($\hat{s}_{ij}$) for popping expansion (PE) in diallel hybrids of popcorn evaluated under high and low phosphorus availability in Itaocara. (−) signal indicating negative values.

Fifty percent of the 90 hybrids evaluated for $\hat{s}_{ij}$ displayed crosses with dominance gene effects in the environment with optimal phosphorus availability, whose combinations with the highest expressions of heterosis were: L684 × L54, L692 × L54, and L684 × L7. As for the environment with low phosphorus level, 47.80% of the combinations exhibited positive values. Of these, the hybrids that performed best were L694 × L54, L685 × L70, and L684 × L80, with the highest values of the specific combining ability effects (Figure 6).

Regarding the genetic merit of the hybrids tested in the environments, it was found that the combination L688 × P7 presented favorable alleles for the highest expression of PE, in addition to having good phenotypic plasticity, since it ranked well in both growing conditions. Among the hybrids with the best performance in the environment with phosphorus supplementation, the following combinations can be considered: L688 × P7, L696 × P7, and L690 × L80. As for the environment without a phosphate fertilizer supply, the hybrids ranking highest were L91 × P7, L685 × L70, and L688 × P7 (Figure 6). The good performance of the tester P7, which was in the best hybrid combinations and expressed the highest $\hat{g}i$ estimates in both high and low P level environments, should again be noted.

In the evaluation of PV in Campos dos Goytacazes, 56.70% of the combinations were found to have positive estimates of $\hat{s}_{ij}$ in the environment with phosphorus supplementation. The combinations L689 × L59, L691 × P7, and L686 × L80 expressed the highest estimates of dominance gene effects. Such results corroborate the effects seen for GY when its SCA was evaluated. This result is understandable, since PV is a combination of GY and PE (Figure 7).

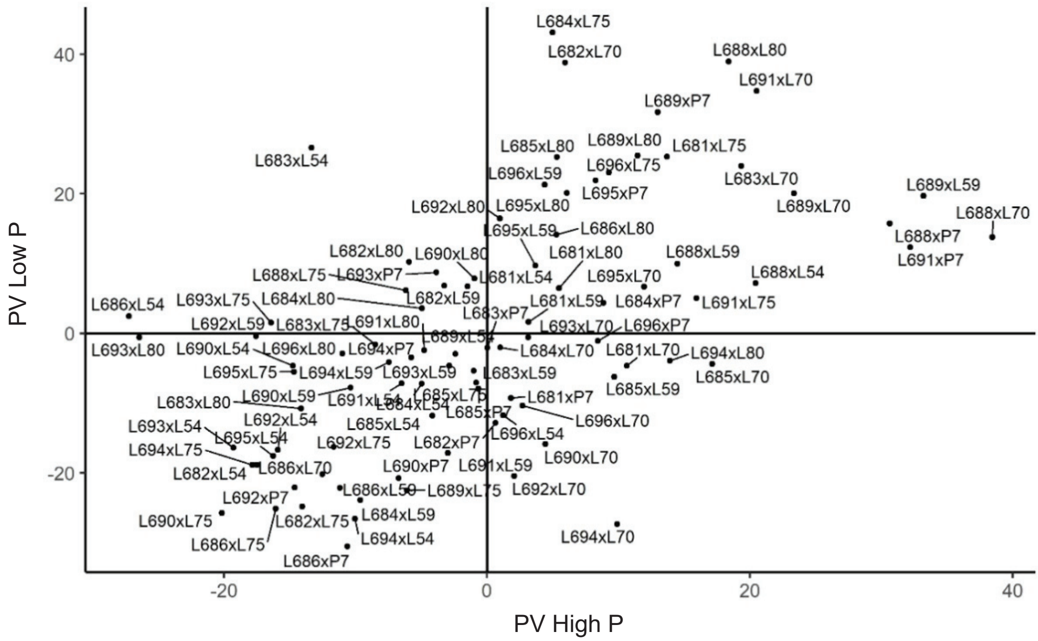

Figure 7. Estimates of the genotypic value ($\hat{s}_{ij}$) for expanded popcorn volume per hectare (PV) in diallel hybrids of popcorn evaluated under high and low phosphorus availability in Campos dos Goytacazes. (−) signal indicating negative values.

Regarding the best tester for this index, line L70 provided the highest heterosis in the crosses, totaling 21.56% of the combinations with positive values. This tester, along with line L80, showed the highest additive gene effects of $\hat{g}_i$ for this trait. In the environment without the nutrient supplementation of phosphorus, the most frequent tester in the crosses with good allelic complementation was the parent L80 when evaluating its SCA estimates, which comprised 25% of the crosses with higher magnitudes of SCA. This result corresponds with the additive gene effects assigned to this genotype when its $\hat{g}_i$ estimate was analyzed. As for the hybrids with the highest SCA estimates, they were L684 × L75, L682 × L70, and L691 × L70, respectively, when considering the 36 crosses with the highest magnitudes of dominance effect (Figure 7).

The genetic merit of the hybrids was also quantified for the PV index of plants grown in both environments in Campos dos Goytacazes. The evaluations indicated that the most vigorous combinations corresponded to L684 × L75, L682 × L70, and L688 × L80, in the nutrient-stressed environment, and L688 × L70, L689 × L59, and L691 × P7, in the experiment with a phosphorus supply. It should be noted that line L688 and tester L70 exhibited excellent performances per se when analyzing their respective general combining abilities, corroborating the prevalence of additive gene effects in both gene conformations (Figure 7).

The PV supercharacter also allowed for the discrimination of the behavior of the 90 hybrids evaluated in Itaocara. The combinations L694 × L80, L684 × L75, and L685 × P7 stood out with the highest heterosis in a set of 49 hybrids, for which there was a prevalence of dominance gene effects in the environment with phosphorus supplementation. The most heterotic hybrid—L694 × L80—is assumed to have the highest divergence among the parents (Figure 8).

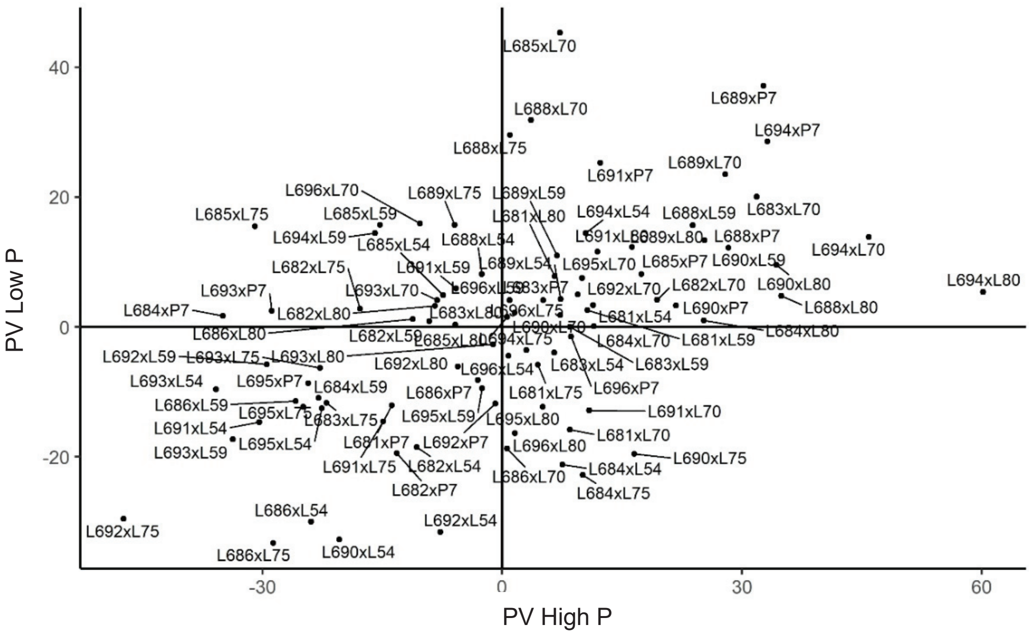

Figure 8. Estimates of the genotypic value ($\hat{s}_{ij}$) for expanded popcorn volume per hectare (PV) in diallel hybrids of popcorn evaluated under high and low phosphorus availability in Itaocara. (−) signal indicating negative values.

In the low-phosphorus environment, 47 hybrids expressed dominance gene effects, with their amplitudes ranging from 0.255 to 21.709 for estimates of $\hat{s}_{ij}$. After selecting the hybrids with positive values, the ones with the highest heterosis were determined: L685 × L70, L691 × P7, and L688 × L75, respectively. It should be mentioned that tester L70, which comprised the best hybrid, was also found in a greater number of positive combinations for both environments (Figure 8). Therefore, it may be assumed that the higher frequency of this parent may be related to the additive gene effects attributed to it when considering its $\hat{g}_i$ estimate.

The evaluation of the combining abilities for PV allowed us to know the genetic merit of the hybrids when grown in Itaocara. This analysis pointed out that the combinations L694 × L80, L694 × L70, and L688 × L80 had the best performances for the high-phosphorus-availability conditions. Hybrids L685 × L70, L689 × P7, and L688 × L70 were also identified as the ones with the best performance in the low-phosphorus environment. Moreover, using the evaluations conducted for PV, it could be verified that there was a predominance of the L70 and L80 testers in the low- and high-nutrient-availability environments. Additionally, the combinations L689 × P7 and L694 × P7 presented the greatest phenotypic plasticity since their recommendations may be made in either an environment with low or high phosphorus levels (Figure 8).

3. Discussion

The genetic variability among the lines under study due to the significant differences among the genotypes for the traits investigated—GY, PE, and PV—in Campos dos Goytacazes and Itaocara is evident (Table 1). Furthermore, the source of variation in phosphorus (P) availability also had significant effects for all the traits; this suggests that the dose of phosphorus applied to the soil was adequate to provide a distinction between the environments and enable correct differentiation of the efficient genotypes from the inefficient ones in phosphorus use in Campos dos Goytacazes and in Itaocara. This points out that the line classification differed between the two growing conditions (high and low phosphorus levels in the soil), indicating a specific P-related effect. This provides strong evidence that there was significant variation in the ability of the material investigated to explore P in its environment, a prerequisite for selection-based breeding. However, screening this performance is not informative with respect to the number of genes underlying the variation observed, but encourages further research focusing on QTL analysis to provide more information on the genetic architecture of variation in response to different P levels in soil [27].

In addition to this, genetic variability in germplasm collections is an essential factor in obtaining genetic gains in breeding programs. Due to the high consumption of phosphate fertilizers—which may lead to a shortage of P reserves within a few decades—and the need to develop sustainable agriculture, these genotypes have become important sources of tolerance for breeding programs of popcorn to obtain gains in efficiency and responsiveness in phosphorus use. Gerhardt et al. [10] also reported genetic variability for agronomic traits when evaluating popcorn hybrids and lines at contrasting phosphorus levels in soil.

The G $\times$ P interaction, in turn, was significant for all the traits, suggesting a dissimilar response of the popcorn hybrids under different phosphorus-availability conditions (Table 1). This interaction may provide changes in the classification of hybrids between the experiments with high and low phosphorus levels. In this regard, Vencovsky and Barriga [28] recommend that the practice of genotype selection should be environment-specific, that is, each environment should have a set of distinct or partially distinct genotypes. Thus, selection should not be made based on average performance, as favorable alleles that control the expression of the character under stress differ, at least partially, from favorable alleles that control the same character under optimal conditions [29].

Significance in the estimates for all variables, except for PV, was observed in Campos dos Goytacazes when the effects of the source genotype variation were unfolded into general combining ability (GCA) and specific combining ability (SCA). This demonstrates that by exhibiting significance for GCA and SCA, GY and PE showed variability resulting from additive and non-additive effects in controlling gene expression. The significance of variation attributed to the additive effects proves that there are promising parents to be used, mainly in intrapopulation breeding programs, as this variation is very useful in pre-breeding to incorporate exotic germplasms into adapted populations or to adapt populations to abiotic stressors (Table 1). The magnitude of the additive variance expressed by the mean squares of the GCA of the progenies and the testers indicates the existence of significant additive effects of the genes [28].

Regarding the effects of specific combining ability—highly significant for the variables GY, PE, and PV ($p \leq 0.01$) in Campos dos Goytacazes—it may be assumed to be the gene action of dominance in the expression of PE, which is a trait known to be governed by additive effects, as reported by Dofing et al. [30], Pacheco et al. [31], Larish and Brewbaker [32], and Pereira and Amaral Júnior [15]. As for Itaocara, there was only significance for GY at a 5% probability level (Table 1). This indicates that non-additive gene effects also exert an influence on these traits and suggests possible allelic complementation between the parents at the respective loci, with some degree of dominance. This possibility for PE gene expression agrees with more recent results obtained by Coan et al. [33], who support the existence of mixed inheritance in the expression of this trait.

In view of the significant progeny interaction with contrasting phosphorus levels (GCA I $\times$ P) for the traits GY and PE in Campos dos Goytacazes, and for the traits GY, PE and PV in Itaocara, it may be assumed that the additive gene effects between the lines provided differences. In this case, then, selection for each level of phosphorus is recommended, as already mentioned. The tester interaction with phosphorus levels (GCA II $\times$ P), also significant for all the traits evaluated, suggests that selection should be made for each phosphorus level. Gerhardt et al. [10], likewise, found significance for the interaction between popcorn progenies and contrasting environments in using phosphorus.

From the results, considering the significant interaction between specific combining ability and phosphorus levels (SCA $\times$ P), it can be noticed that the classification of SCA effects differed between environments. The results at each fertilization level should, thus, be considered to allow for the effective selection of hybrids that are efficient and responsive to phosphorus use.

As for the experimental precision expressed by the coefficient of experimental variation (CVe), the values found for all the traits were less than 20%, suggesting excellent experimental precision [34]. These results agree with the estimates found by Santos et al. [35] (2017) and Gerhardt et al. [10] in experiments with abiotic stressors in popcorn.

According to Sprague and Tatum [36], informed by Inocente et al. [37], the GCA corresponds to the average behavior of a line in a series of hybrid combinations, and is expressed by the $\hat{g}i$ estimate. For Cruz and Vencovsky [38], a low value of $\hat{g}i$ suggests that the average of the hybrids in which line *i* participates does not differ much from the overall average of the diallel, meaning that when there are high values, positive or negative, parent *i* is superior or inferior to the other parents in the diallel when compared to the average of their hybrids. Thus, the estimates of the $\hat{g}i$ effects for the variables analyzed have values with signs ranging from negative to positive as a function of the performance of the parent. Accordingly, Cruz and Vencovsky [38] and Scapim et al. [39] affirm that the line with the highest frequency of favorable alleles will express a higher $\hat{g}i$.

Based on these references and considering the $\hat{g}i$ estimates, it is observed that, in Campos dos Goytacazes, lines L682, L688, and L686 had the three highest values—338.431, 310.452, and 290.454, respectively—in the environment with adequate phosphorus availability, and, in the environment with low phosphorus levels, the most positive values were assigned to the genotypes L688 (313.311), L695 (280.976), and L689 (207.294) (Figure 1).

Line L688 was not affected by a substantial reduction in phosphorus in the soil; thus, its high combining ability and phenotypic stability are relevant for programs aimed at increasing GY. Scapim et al. [39] reported the use of popcorn populations with high GCA estimates to form 211 varieties with high GY and PE, supporting the relevance of working with parents with high $\hat{g}i$ estimates for these traits, which are both of major relevance for popcorn trading.

Regarding the testers, for GY, under optimal phosphorus supply conditions, three lines showed positive deviations (L70, L59, and L75) and four stood out for the environment with induced stress (L80, L59, L70 and L75). Among the best lines used as testers, three—L70, L59, and L75—had positive values in both conditions of phosphorus availability. It should be emphasized that tester L59 had the least variation (367 and 739), ranking well in both phosphorus conditions in the soil, in Campos dos Goytacazes. This good performance may be associated with its genealogy (Beija-flor: UFV) and adaptation to a temperate/tropical climate, a factor correlated with the presence of favorable alleles in a good parent (Figure 1).

For PE, eight lines under optimal conditions of phosphorus availability and seven lines under limiting conditions of the nutrient showed positive values for $\hat{g}i$ estimates in Campos dos Goytacazes. There is agreement in the prominence of lines L681, L689, L690, L681, L688, and L689 (Figure 1) in both conditions of phosphorus availability; however, lines L681, and L689 had the greatest $\hat{g}i$ effects and least variation between the two environments. Therefore, it may be recommended that these parents obtain lines with good PE, since $\hat{g}i$ is the estimator that indicates the parents with the best average performance in crosses. These lines, thus, have the highest concentrations of favorable alleles for traits predominantly influenced by additive gene action.

As for the testers that were outstanding in environments with high (P7 and L54) and low (P7, L80, and L54) phosphorus levels, P7 showed the best performance regardless of phosphorus availability in the soil; this represents a line of interest to be included in crosses aimed at obtaining superior hybrids in relation to efficiency and responsiveness in phosphorus use.

Kamphorst et al. [40] further reported that line P7 showed high GY grain yield averages when grown in a water-stressed environment, and were considered agronomically efficient in water use. This suggests that this line has mechanisms to withstand adverse situations imposed by abiotic stress. Santos et al. [20] also described the relevance of P7 for GY and PE traits under optimal and low soil-nitrogen-availability conditions from a panel of ten popcorn lines evaluated in Campos dos Goytacazes and Itaocara. When evaluating 25 popcorn lines under high and low P conditions in soil, Gerhardt et al. [10] pointed out that, because of high $\hat{g}i$ estimates for GY and PE, line P7 has high potential for obtaining superior hybrids in terms of efficiency and responsiveness in phosphorus use.

In Campos dos Goytacazes, six lines distinguished themselves, for PV, by having positive values of $\hat{g}i$ estimates in the environment with high phosphorus levels—L688, L689, L691, L681, L685, and L696—and, in limiting conditions of the nutrient, eight lines stood out with positive values, namely: L688, L689, L695, L683, L681, L691, L696, and L684. When both environments were analyzed, it was noted that the lines with the best performance were L688 and L689, respectively (Figure 1). This coincidence of results corroborates the good performance of these genotypes and the substantial incidence of favorable alleles for phosphorus efficiency and use in the lines derived from the UENF-14 population (Table 1). The PV trait is considered a supercharacter that is intended to simulate a selection index, which enables concomitant gains for the two main traits of economic importance—GY and PE expansion—for the popcorn crop [41].

Still considering the PV, among the testers investigated, lines L70, P7, and L59 prevailed in the environment of high phosphorus levels in the soil, having shown positive $\hat{g}i$ results, whereas in phosphorus-limiting conditions, only two stood out—L80 and L70. Among the genotypes with good results, tester line L70, from the BRS Angela population, distinguished itself by having positive values for PV at both phosphorus levels in Campos dos Goytacazes (Figure 1). These results agree with the work of Schmitt et al. [42], who reported the good performance of the parent L70 in presenting a positive $\hat{g}i$ estimate for PE.

As for the effects of GCA for GY, in Itaocara, eight lines were highlighted based on $\hat{g}i$ estimates in the environment with high phosphorus level. Similarly, in low P conditions, eight lines also stood out. Considering both conditions, however, five lines distinguished themselves in both environments by presenting positive deviations, as follows: L694, L689, L682, L684, and L688. Within them, lines L694 and L689 were highlighted in the ranking, in that order (Figure 2).

When it comes to the $\hat{g}i$ effects of the testers, still for GY in Itaocara under high phosphorus conditions, three lines were prominent, and four genotypes were superior under P-deficient conditions in the soil. When analyzing both conditions of phosphorus availability, tester L70—which has adaptations to tropical environments—displayed greater stability, only surpassed by lines L80 and L59—adapted to temperate and tropical climates—which were superior, respectively, in high- and low-P environments. This suggests good adaptation of these genotypes to the conditions studied for GY. By evaluating 15 populations of popcorn from different Latin-American countries under water-stress conditions, Santos et al. [43] related the importance of varieties with temperate and tropical climatic adaptations. Thus, according to the authors, the relevance of these materials in studies with popcorn to obtain superior genotypes is reinforced. Gerhardt et al. [10] pointed out, in a previous study, line L59 as being efficient and responsive in the use of phosphorus. Therefore, it is a genotype that expresses superior yields to the averages in environments of high and low phosphorus levels.

In this context, it is verified that, for the best S_7 progenies, as well as for the testers, the $\hat{g}i$ estimates for PE were negative. This is due to the negative genetic correlation between GY and PE, a phenomenon already reported in other research conducted in the popcorn crop [15,16,30,43–47]. This correlation, therefore, makes it difficult to obtain genotypes with high GY and high popping expansion concomitantly [15,48,49].

By analyzing the results of the $\hat{g}i$ estimates for PE in Itaocara, even with the complex interaction, the best-performing genotypes, under optimal phosphorus conditions in the soil, were also better under limiting conditions. In this case, there were only differences in the ranking order, indicating that the change in the crop environment caused alterations in the performance of the genotypes under different P conditions. As stated before, when the interaction is of the complex type, it makes it difficult to indicate genotypes for a group of environments, requiring the breeder to recommend appropriate genotypes for specific conditions. The performance of lines L681 and L688 proved to be encouraging for this study, since they showed good phenotypic plasticity for the trait in question, ranking well in both environments, regardless of the P supply. Regarding the testers, the positive results of lines P7, L70, and L80 in both environments distinguish them as potential candidates for obtaining superior hybrids in both environments.

With regard to the $\hat{g}i$ effects for PV in Itaocara, lines L694, L688, and L689 were superior to the others as they showed positive values under optimal phosphorus conditions and when under nutrient stress, not exhibiting significant differences in performance, and keeping the positive values for $\hat{g}i$. For the testers, L70 showed the same behavior, performing well under both phosphorus-availability conditions.

Given these considerations, it may be stated that the UENF-14 popcorn population has a high frequency of alleles favorable for the efficiency of phosphorus use in the soil. This assertion is supported by the good performance of lines L688 and L689, which showed good general combining ability and high GY, as well as high popping expansion, suggesting that there is a distinct heterotic pattern. Thus, lines L688 and L689 and testers P7 and L59 are highly recommended for use in future hybrid combinations aimed at increasing GY and PE in environments with low soil-phosphorus levels in breeding programs focusing on more sustainable agriculture.

By considering the merit of the hybrids for the GY and PV traits together, it was assumed that the L688 × L70 cross had higher heterosis when evaluated in environments with high P levels in the soil. In the environment with stress induction, heterosis was more evident in the genotype L684 × L75. Therefore, the interaction of these genotypes with the environments provided relative superiority compared to the others, suggesting that there are favorable alleles in these hybrid combinations.

For the traits PE and PV together, changes in the ranking of the crosses were observed, highlighting the superiority of the genotype L689 × L59, which showed the highest heterosis in the environment with high phosphorus levels. Thus, the hybrid combination L689 × P7 may be considered the most relevant one for the low-phosphorus environment in the experiments in Campos dos Goytacazes. As for Itaocara, the hybrid combinations with the highest popping expansion were L694 × L80 in the environment with added phosphorus, and L685 × L70 in the environment with low phosphorus levels. In relation to the testers, lines P7 and L54 showed additive genetic effects for GCA in the deficient environments, increasing popping expansion. The other testers distinguished themselves through their good allelic complementation in the crosses in which they participated.

When considering GY and PV traits together in Itaocara, the hybrid L694 × L80 was chosen as the most prominent for the environment with fertilizer recommendation for popcorn. For the low-phosphorus environment, the combination with the highest heterosis was L685 × L70; this hybrid also showed positive a magnitude of $\hat{s}_{ij}$ when its SCA was evaluated.

More generally, the genotypes utilized present genetic potential for obtaining hybrids with high heterosis for efficiency and responsiveness in phosphorus use. Considering the prevailing gene effects of the GY, PE, and PV traits in the contrasting environments, the hybrid combinations that displayed promising results for efficiency and responsiveness in phosphorus use were L688 × L70, L694 × L80, L688 × L70; L694 × L80, L689 × L59, L694 × L80; and L689 × P7, and L689 × L59, respectively.

From the information obtained by means of the combining ability and having the most promising combinations, the parents from the UENF-14 population with the best performance—L688, L694, and L689—may be selected as standards for the creation of a new heterotic group and as testers of new lines from this heterotic group. Thus, this new heterotic group should be maintained and used separately for the generation of variability within the group, and for the selection of new lines.

Accordingly, the implementation of a procedure called "line recycle", originating from the UENF-14 population, is also proposed; this will form new biparental populations directly or via backcrossing, in which the elite lines L688, L694, and L689 from the UENF popcorn breeding program will be crossed among themselves, in subsequent cycles, within this same heterotic group. Thus, new lines will be obtained with superior traits to their parents because of the increased frequency of favorable alleles and the consequent higher level of heterosis when crossed to form a heterotic group.

In conclusion, to meet the agroeconomic aspects of the North and Northwest regions of Rio de Janeiro State—which tend to expand—on the basis of the combining ability and of the presence of favorable alleles, we propose obtaining a triple hybrid derived from a simple hybrid from parents originating from the heterotic group of the UENF-14 population—L688 $\times$ L689, for example, whose parents showed high GCA; this will be used as the female parent in a cross with the L80 line. Such a line has been demonstrated to be sufficiently vigorous to ensure good pollination and, consequently, satisfactory GY in female plants.

4. Materials and Methods

4.1. Plant Material, Experimental Design, and Environmental Conditions

Six S_7 lines from the Germplasm Bank of the *Universidade Estadual do Norte Fluminense Darcy Ribeiro* (UENF)—previously classified by Gerhardt et al. [50] regarding P use—were used as testers, of which three (L59, L70, and P7) were efficient and responsive and three (L54, L75, and L80) were inefficient and non-responsive. They were used as testers in crosses with 15 other progenies (L681, L682, L683, L684, L685, L686, L688, L689, L690, L691, L692, L693, L694, L695, and L696) (Table 2) from the UENF-14 open-pollinated variety. This population was selected after five cycles of intrapopulation recurrent selection, adapted to the soil and climate conditions of the North and Northwest regions of Rio de Janeiro State [51]. Ninety testcrosses were generated from the crosses in a partial diallel scheme of the six testers with the 15 UENF-14 progenies.

Table 2. Description of the 21 popcorn genotypes from the Active Germplasm Bank of UENF used in the experiments in the North and Northwest regions of Rio de Janeiro State, Brazil.

Tester	Genealogy	Climate Adaptation	Classification Regarding P Use
L59	**Beija-Flor: UFV**	**Temperate/Tropical**	**Efficient and Responsive**
L70	**BRS Angela: EMBRAPA**	**Tropical**	**Efficient and Responsive**
P7	Híbrido Zaeli	Temperate/tropical	Efficient and responsive
L54	Beija-flor: UFV	Temperate/tropical	Inefficient and non-responsive
L75	Viçosa: UFV	Temperate/tropical	Inefficient and non-responsive
L80	Viçosa: UFV	Temperate/tropical	Inefficient and non-responsive

IRS Progenies		Genealogy and Climate Adaptation		IRS Progenies		Genealogy and Climate Adaptation		IRS Progenies		Genealogy and Climate Adaptation	
1	L681	UENF-14	Tropical	6	L686	UENF-14	Tropical	11	L692	UENF-14	Tropical
2	L682	UENF-14	Tropical	7	L688	UENF-14	Tropical	12	L693	UENF-14	Tropical
3	L683	UENF-14	Tropical	8	L689	UENF-14	Tropical	13	L694	UENF-14	Tropical
4	L684	UENF-14	Tropical	9	L690	UENF-14	Tropical	14	L695	UENF-14	Tropical
5	L685	UENF-14	Tropical	10	L691	UENF-14	Tropical	15	L696	UENF-14	Tropical

UFV—Universidade Federal de Viçosa; UENF—Universidade Estadual do Norte Fluminense Darcy Ribeiro; EMBRAPA—Brazilian Agricultural Research Corporation; and IRS—Intrapopulation Recurrent Selection.

The experiments were conducted in a randomized block design with replication arrangements within sets. Five sets of 18 treatments (15 progenies plus three controls: UENF N 01, UENF N 02, and UENF HS 03) in each set were utilized, with three replications. These experiments were carried out during the harvest period, between October 2019 and March 2020, in two locations and under two contrasting conditions with respect to phosphorus availability (high and low phosphorus). The sites were the Experimental Station of the Colégio Estadual Agrícola Antônio Sarlo, in the municipality of Campos dos Goytacazes (latitude: 21°42′48″ S, longitude: 41°20′38″ W, 14 m above sea level), and the Experimental Station of Ilha do Pomba, in the municipality of Itaocara (latitude: 21°38′50″ S, longitude: 42°03′46″ W, 58 m above sea level); these correspond, respectively, to the Northern and

Northwestern regions of Rio de Janeiro State. The climate in Campos dos Goytacazes and Itaocara is classified as humid tropical (Aw), with hot summers and mild winters, with rainfall tending to be concentrated in the summer months.

Sowing was performed according to the conventional planting system, with a stand of 15 plants per plot, or 55,555 plants per hectare. Each experimental plot consisted of a 3.00 m row with 0.90 m spacing between rows and 0.20 m spacing between plants.

Prior to the experiments, soil chemical analysis was conducted to characterize the environments in terms of nutrient availability from samples collected in the 0–10 and 10–20 cm layers, forming a sample composed of ten subsamples (Table 3). The available P content was determined using the Mehlich-1 extractor. According to the clay soil content of Campos dos Goytacazes and Itaocara, the phosphorus levels were classified as low [52] (Ribeiro et al., 1999).

Table 3. Chemical and particle-size analysis of the soil at 0–10 and 10–20 cm depths in Campos dos Goytacazes and Itaocara.

Local	Soil Layer	pH	P	K	Ca	Mg	Al	Na	C	OM	CEC	BS	V	Clay
		H_2O	mg/dm^3	mmol$_c$/dm^3					g/dm^3		mmol$_c$/dm^3		%	g/dm^3
Campos dos Goytacazes	0–10 cm	5.7	4.0	3.4	14.6	8.3	0.0	1.3	7.6	13.1	48.9	27.6	56.0	305.0
	10–20 cm	5.4	3.0	2.3	14.4	6.7	0.9	1.2	7.2	12.4	44.5	24.6	55.0	
Itaocara	0–10 cm	5.2	5.0	1.2	38.9	30.0	1.2	0.6	14.9	25.7	103.8	70.7	68.0	140.0
	10–20 cm	5.3	3.0	0.7	36.2	31.0	1.4	0.4	11.0	19.0	96.2	68.3	71.0	

Two doses of this fertilizer were used to simulate contrasting environments and to stimulate the genotypes to express the genes responsible for phosphorus efficiency and responsiveness. In the environment with high phosphorus availability, 30 kg ha^{-1} of N (in the form of urea), 85 kg ha^{-1} of P_2O_5 (triple superphosphate), and 40 kg ha^{-1} of K_2O (potassium chloride) were applied. For the environment with low phosphorus availability, 30 kg ha^{-1} of N, 0 kg ha^{-1} of P_2O_5, and 40 kg ha^{-1} of K_2O were used, which means the fertilizer in the stressed environment did not contain phosphorus. Topdressing fertilization was performed in partial applications in both environments when the plants reached the phenological stage of four (V4) and six (V6) fully expanded leaves at a concentration of 100 kg ha^{-1} of N (in the form of urea). The other phytosanitary treatments were performed according to the recommendation for the crop in the North and Northwest regions of Rio de Janeiro State [53]. The experiments received additional irrigation whenever necessary to avoid water stress.

4.2. Evaluated Traits

The following traits were evaluated: (i) Grain yield (GY)—expressed by the average grain yield of the experimental unit in grams per plot, adjusted to 13% moisture and extrapolated to kg ha^{-1}. (ii) Popping expansion (PE)—obtained using the ratio between the expanded popcorn volume and the mass of 30 g, expressed in mL g^{-1}, utilizing the average of two samples per plot. Popping was conducted in a microwave oven, with 1200 Watts of power for 2 min, and the expanded popcorn volume was quantified in a 2000 mL beaker. The resulting value was divided by the initial weight of the 30 g grain, and the final result expressed in mL g^{-1}. (iii) Expanded popcorn volume per hectare (PV), determined using the product between GY and PE, with the final value divided by 1000 and expressed in m^3 ha^{-1}.

4.3. Data Analysis

Adjustments of the set effects for all the hybrids were made according to the average of the controls common to all the sets before conducting the analysis of variance. We estimated the average of the controls for each set (ACS) and the general average of the controls (GAC); using the GAC/ACS relationship, the adjustment factor for each set was obtained, following the procedure used by Ribeiro et al. [54] and Guimarães et al. [55].

After these adjustments, the analyses were performed following the randomized block model. At first, the individual analyses of variance were conducted considering the environments with high and low phosphorus, according to the following statistical model: $Y_{ij} = m + B_j + G_i + e_{ij}$, in which Y_{ij} is the observation of the i-th genotype in the j-th block; m is the general constant; B_j is the effect of the j-th block; G_i is the effect of the i-th genotype; and e_{ij} is the experimental error associated with the observation Y_{ij}, which is normally and independently distributed (NID—$0,\sigma^2$).

Subsequently, a joint analysis of variance was conducted to determine possible interactions between the genotypes and the levels of phosphorus in each location. The joint analysis of variance was conducted following the statistical model: $Y_{ijk} = m + B/A_{jk} + A_j + G_i + GA_{ij} + e_{ijk}$, in which Y_{ijk} is the observation of the i-th genotype in the j-th block in the k-th block; m is the general constant; B/A_{jk} is the effect of the k-th block in the j-th environment; A_j is the fixed effect of the j-th environment (P level); G_i is the fixed effect of the i-th genotype; GA_{ij} is the fixed effect of the interaction between the i-th genotype and the j-th environment; e_{ijk} is the experimental error associated with the observation Y_{ijk} with NID ($0, \sigma^2$).

Based on the averages of the 90 testcross hybrids, a diallel analysis was carried out in line with the methodology suggested by Griffing [56], adapted to partial diallels, as follows: $Y_{i\,j} = m + g_i + g_j + s_{ij} + a_k + ga_{ik} + ga_{jk} + sa_{ij} + e_{(k)ij}$, in which Y_{ij}: The average value of the hybrid combination, testers (g_i), and lines (g_j); m: The overall average of the hybrid combinations; g_i: The general combining ability (GCA) effect of group i (progenies); g_j: The GCA effect of group j (testers); s_{ij}: The specific combining ability (SCA) effect for the crosses between parents of the orders i and j; a_k: The effect of the environments k; ga_{ik} and ga_{jk}: The interaction effects between the GCA associated with the i-th and j-th parents in the environments k; sa_{ij}: The interaction effect of SCA associated with parents i and j and environments k; and $e_{(k)ij}$: The average experimental error.

The statistical analyses were conducted using the software GENES (v.1, Universidade Federal de Viçosa, MG, Brazil) [57].

5. Conclusions

Phosphorus use efficiency is mostly dependent on additive gene effects in the expression of popping expansion, and conversely, dominance gene effects in the expression of GY and PV.

The best strategy to obtain efficient and responsive genotypes in phosphorus use involves the exploration of heterosis, with parents that provide an accumulation of additive genes for popping expansion.

Lines L688 and L689 and testers P7 and L59 showed additive gene effects and are highly recommended for use in future hybrid combinations looking to increase GY and PE in environments with low soil-phosphorus levels.

The genetic merit of the hybrids enabled us to know the combinations with the highest dominance gene effects for efficiency and responsiveness in phosphorus use for the traits GY, PE, and PV, as follows: L688 × L70, L694 × L80, L688 × L70; L694 × L80, L689 × L59, L694 × L80; L689 × P7, and L689 × L59, in that order.

Due to the good performance of the parents originating from the UENF-14 population, L688, L694, and L689 are selected as the standards for forming a new heterotic group and for "line recycle", creating new biparental populations.

The production of a triple hybrid by crossing the simple hybrid L688 × L689—from the UENF-14 heterotic group—with the L80 line—from the Viçosa: UFV genealogy—is a good alternative for meeting the needs of the North and Northwest regions of Rio de Janeiro State and for achieving sustainable agriculture.

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plants

Article

Chloride Applied via Fertilizer Affects Plant Nutrition and Coffee Quality

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Abstract: The present study had the objective to evaluate the effect of blends of KCl and K_2SO_4 fertilizers and their influence on the yield and the nutritional state of coffee plants, as well as on the chemical composition and quality of the coffee beverage. The experimental design was in randomized blocks with four repetitions and six treatments (T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; and a control, without application of K). The following analyses were performed: K and Cl content in the leaves and the soil, stocks of Cl in soil, yield, removal of K and Cl with the beans, cup quality of the beverage, polyphenol oxidase activity (PPO), electric conductivity (EC), potassium leaching (KL), the content of phenolic compounds, the content of total sugars (TS), and total titratable acidity (TTA). The stocks of Cl in the soil decreased as the proportion of KCl in the fertilizer was reduced. The fertilization with KCl reduces the cup quality and the activity of the polyphenol oxidase, probably due to the ion Cl. The increase in the application of Cl directly relates to the increase in potassium leaching, electric conductivity, and titratable acidity. Indirectly, these variables indicate damages to the cells by the use of Cl in the fertilizer. The activity of the polyphenol oxidase enzyme and the cup quality indicate that the ion Cl- reduces the quality of the coffee beverage. K content in the leaves was not influenced by the application of blends of K fertilizer while Cl content increased linearly with KCl applied. The application of KCl and K_2SO_4 blends influenced coffee yield and the optimum proportion was 25% of KCl and 75% of K_2SO_4. The highest score in the cup quality test was observed with 100% K_2SO_4.

Keywords: blend fertilizers; chlorine; cup test; polyphenol oxidase

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1. Introduction

Coffee is one of the most popular beverages in the world, and its cultivation is widespread in 80 countries. Brazil is the largest producer and second largest consumer of coffee in the world. The gross revenue of "Cafés do Brasil" in the 2022 harvest was R$61.82 billion with *Coffea arabica* accounting for 77% the total revenue (R$ 47.48 billion). The state of Minas Gerais is the largest producer, responding for R$ 33.28 billion or 54% of national revenues [1]. After petroleum, coffee is the second most commercialized product [2,3]. The marketing price is based on the quality of the beverage, which is related to the physical, chemical, and sensorial characteristics of the product [2,4,5].

Fertilization and crop nutrition can influence both yield and the chemical composition of the raw beans, which, consequently, interfere with the quality of the beverage [6].

After nitrogen, potassium (K) is the most accumulated nutrient in coffee plant fruits, where it is demanded in high amounts. K is related to the enzymatic activation of several metabolic processes, such as photosynthesis, proteins, and carbohydrates synthesis, and in the maintenance of cell turgidity [7–9]. In addition, K is directly related to the transport of sugars from the source to the drain (fruits) [10].

The effects of the accompanying chloride ion (Cl) of the potassium chloride fertilizer (KCl) are currently under debate. Cl is demanded by the plants at low amounts, thus being one of the last micronutrients to enter the micronutrient list. Its role is related to the water photolysis on the photosystem II, enzyme activation (amylase, asparagine synthetase, and tonoplast ATPase), and stomatal control [11].

Despite Cl being essential to plant nutrition, when accompanying a highly demanded macronutrient such as K, it can reach excessive concentrations in the soil and plants [12] and consequently reduce the quality of the beverage. In coffee plants, high concentrations of Cl are related to the increase in plant water, which favors an undesirable fermentation of the fruits by microorganisms [13,14].

The study of the Cl influence on the quality of the coffee beverage is not recent, but it is still inconclusive. For example, Dias et al. [15] evaluated an alternative source of K (glauconite silicate mineral) to KCl in coffee fertilization. Despite finding similar yields and polyphenol oxidase activity (PPO) in the beans, the use of the glauconite did not improve the sensorial quality. Silva et al. [16], along two seasons, verified that fertilization with potassium sulfate (K_2SO_4) increased PPO activity in comparison with KCl, which, according to the authors, is indicative of a better beverage quality. These studies suggest possible negative effects of the Cl on the quality of the coffee beverage and the necessity to use K sources without Cl as the accompanying ion, such as K_2SO_4 (48% K_2O, 16% S) and potassium nitrate (44% K_2O, 13% N). Nonetheless, this could increase the production costs, as these sources are more expensive than KCl. In turn, blends of KCl and K_2SO_4 (a physical mixture of the two less expensive sources in the market) could be an alternative to reduce Cl to thresholds that do not affect the quality of the coffee beverage without excessively increasing the costs of the fertilization.

Therefore, the present study had the objective to evaluate the effect of blends of KCl and K_2SO_4 fertilizers at different proportions and their influence on the yield and the nutritional state of coffee plants, as well as on the chemical composition and quality of the coffee beverage.

2. Results

2.1. *Effects of the KCl and K_2SO_4 Blends in the Stocks of Cl in the Soil, Nutrition, and Yield of Coffee Plants*

2.1.1. Harvest of 2017/2018

The initial content of K in the 0–20 and 20–80 cm layers was 91.5 and 58.6 mg dm^{-3} while stocks of the element were 201.3 and 386.7 kg ha^{-1}, respectively. The content of Cl in the 0–20 and 20–80 cm layers was 153.8 and 203.8 mg dm^{-3} while stocks were 338.3 and 1345.0 kg ha^{-1}, respectively. K and Cl contents in the leaves were 19.2 g kg^{-1} and 2880 mg kg^{-1}, respectively (Table 1).

Table 1. Initial content and stocks of K and Cl in the soil and in the leaves of coffee plants.

Depth	BD	K^+	Cl^-	$S.K^+$	$S.Cl^-$	Leaf K^+ Foliar	Leaf Cl^-
cm	g cm^{-3}	mg dm^{-3}		kg ha^{-1}		g kg^{-1}	mg kg^{-1}
0–20	1.1	91.5	153.8	201.3	338.3	19.2	2880
20–80	1.1	58.6	203.8	386.7	1345.0		

BD = Soil density by the volumetric ring method; $S.K^+$ = stocks of K; $S.Cl^-$ = stocks of Cl. Both stocks were calculated by multiplying the content of the element by the mass of soil in the layer.

Soil stocks of Cl were influenced by the K blends (Figure S1). Overall, the amount of Cl decreased along with the KCl proportion in the treatment. In the 0–20 cm layer, Cl stocks with T1, T2, and T3 were similar (~190 kg ha^{-1}). In the 20–80 cm layer, the highest Cl stock was in the T1 (622 kg ha^{-1}), T2 (513 kg ha^{-1}), and T3 (409 kg ha^{-1}; which did not differ from T2). Other treatments showed similar Cl stocks.

In this harvest, K content varied from 20.8 to 34.8 g kg^{-1} (Figure S2). Cl content decreased with the increase in K_2SO_4, with results ranging from 3644 to 5275 mg kg^{-1}. For the statistically non-significant variables, mean values for Cl content in the beans (Figure S3A), yield (Figure S3A), and Cl removal (Figure S3B) were 1778 mg kg^{-1}, 3631 kg ha^{-1} (Figure S3A), and 2.8 kg ha^{-1}, respectively. The lowest K removal by the beans was in T3 (11.3 kg ha^{-1}). The K removal from other treatments had means close to 24 kg ha^{-1} (Figure S3B).

2.1.2. Harvest of 2018/2019

In this harvest, treatments showed Cl stocks near 65 kg ha^{-1} in the 0–20 cm layer (Figure 1). The lowest Cl amount was stocked with T4 (50 kg ha^{-1}). In the 20–80 cm layer, the average stock of the six treatments was 122 kg ha^{-1}.

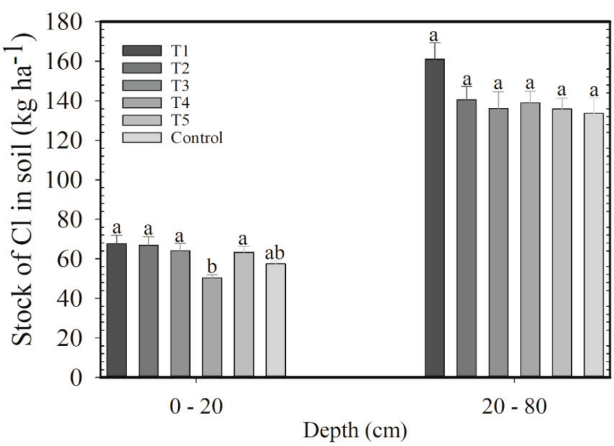

Figure 1. Stocks of Cl in the 0–20 and 20–80 cm layers after application of KCl and K_2SO_4 blends as cover fertilization on coffee plants. 2018/2019 harvest. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

K content in the leaves varied from 17 to 21 g kg^{-1}, and the lowest value was in the control (Figure 2). Overall, Cl content in the leaves decreased with less KCl applied. Treatments T1 (6950 mg kg^{-1}) and T2 (7621 mg kg^{-1}) were far superior from the others.

There was no significant differences among treatments for the following variables: Cl content in beans (693 mg kg^{-1}; Figure 3A), yield (1804 kg ha^{-1}; Figure 3A), and K removal (6.0 kg ha^{-1}) and Cl removal (0.21 kg ha^{-1}; Figure 3B).

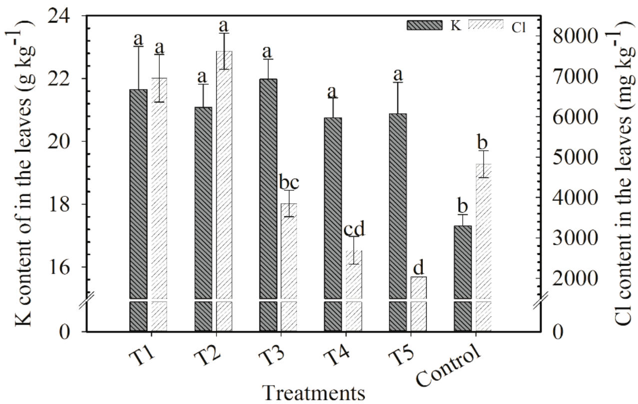

Figure 2. K and Cl contents in the leaves of coffee plants, 20 days after application of the second cover fertilization parcel. Harvest of 2018/2019. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

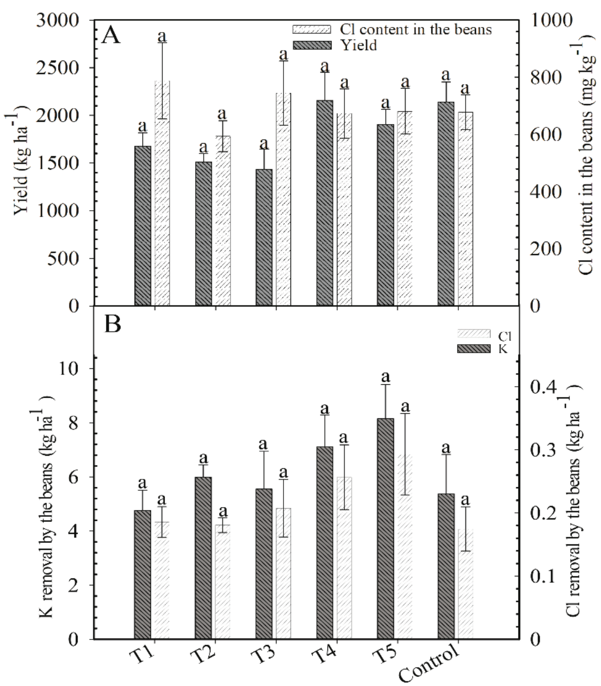

Figure 3. Yield of coffee plants and Cl content in the beans at cherry stage (**A**) and K and Cl removal by the beans (**B**) after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2018/2019. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

2.1.3. Harvest of 2019/2020

The same pattern of the first harvest was observed. Cl stocks in the soil and Cl content in the leaves decreased along with the proportion of KCl in the blend (Figures 4 and 5).

In the 0–20 cm layer, Cl stocks were higher for T1 (119 kg ha^{-1}) and T3 (101 kg ha^{-1}) and lower for T5 (57 kg ha^{-1}) and the control (54 kg ha^{-1}); in the 20–80 cm layer, the lowest values occurred with T5 and control (~267 kg ha^{-1}). The highest Cl content in the leaves was in T1 (4919 mg kg^{-1}), and the lowest content was found in T5 (1762 mg kg^{-1}) and control (1819 mg kg^{-1}).

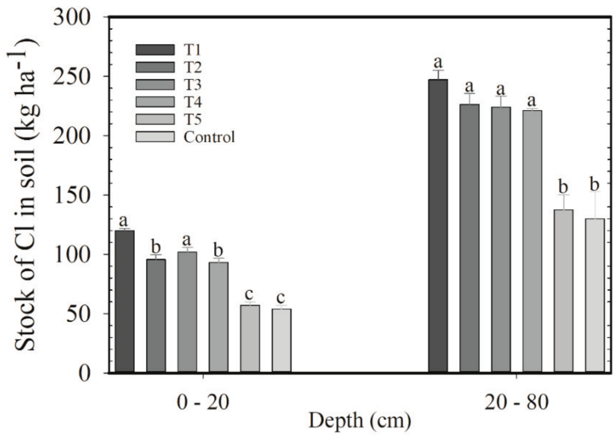

Figure 4. Stocks of Cl in the 0–20 and 20–80 cm layers after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2019/2020. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

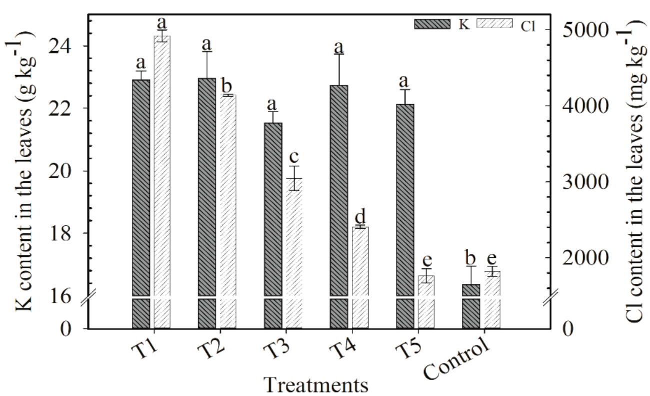

Figure 5. K and Cl content in the leaves of coffee plants. Harvest of 2019/2020. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean (n = 4). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

There were differences in the yield of the coffee plants depending on the treatment, although they all received the same dose of K (Figure 6A). Yields of T1 (4147 kg ha^{-1}) and the control (4055 kg ha^{-1}) were the lowest. The treatments that received K_2SO_4 up to 75% of applied K had similar yields (~5100 kg ha^{-1}). Yields of T2, T3, and T4 were 19, 24, and 24% higher than the yield of T1. Treatment T5 yield was similar to the best yields but did not differ from the yield of T1.

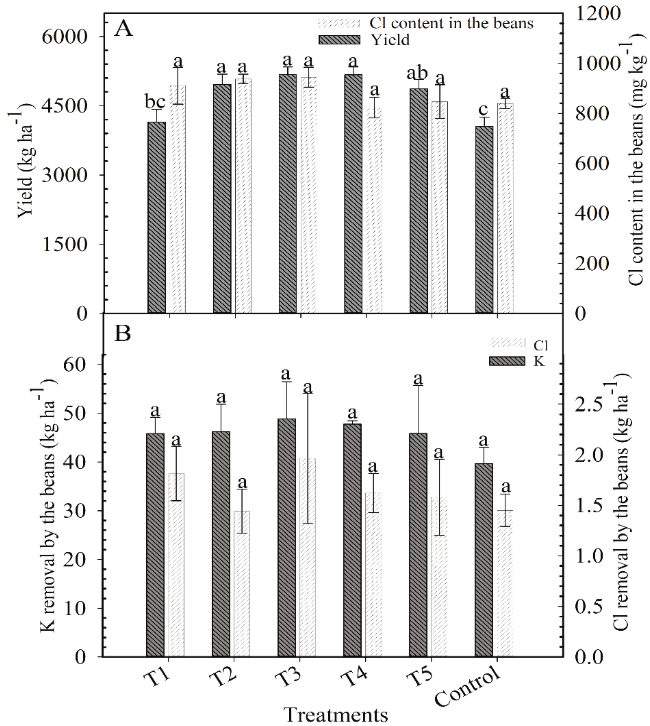

Figure 6. Yield (**A**), Cl content in the beans (**A**), and removal of K and Cl (**B**) by the beans of coffee at cherry stage after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2019/2020. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

There was no significant difference among treatments for the other agronomic variables. The average results were as follows: 883 mg kg^{-1} for the content of Cl in the beans (Figure 6A), 45 kg ha^{-1} for the K removal, and 1.6 kg ha^{-1} for the Cl removal by the beans (Figure 6B).

2.2. *Effect of KCl and K_2SO_4 Blends on the Chemical Composition and Quality of the Coffee Beverage*

2.2.1. Harvest of 2017/2018

The highest K leaching (KL) was in T3 (36.7 µg g^{-1}), and the lowest was in T1 (30 µg g^{-1}) (Figure S4). In the variables related to the quality of the coffee beverage, there was a similar pattern among the treatments despite not having differences from each other. The resulting averages were: 81 points for the sensorial analysis; 91.8 µS cm^{-1} g^{-1} for the electric conductivity (EC); 9.7% for total sugars (TS); 1.04% for caffeine content (Caf); 47.6 u min^{-1} g^{-1} for the activity of polyphenol oxidase (PPO); 186.5 mL NaOH 100 g^{-1} of sample for total titratable acidity (TTA); and 6.4% for polyphenols (Pol) (Table S1).

2.2.2. Harvest of 2018/2019

All treatments received over 80 points in the cup quality (Figure 7A). The highest scores were achieved by treatments T3 (83 points), T4 (84.5), and T5 (83).

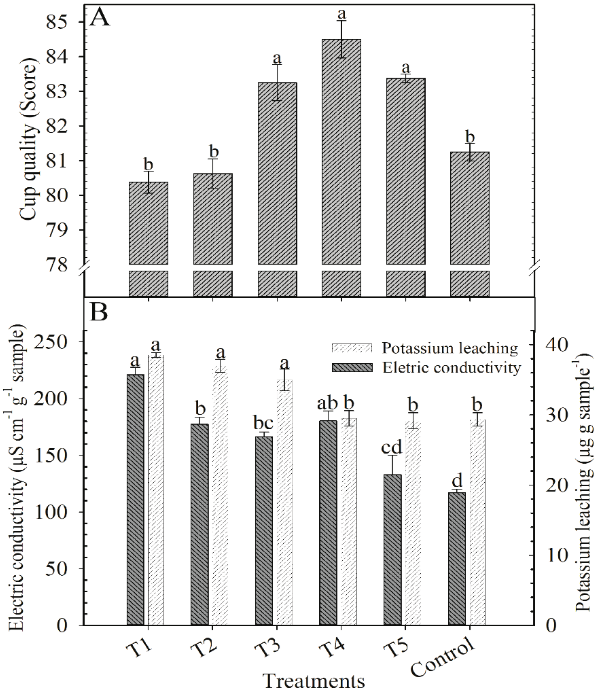

Figure 7. Scores of cup quality (**A**), electric conductivity and potassium leaching (**B**) in coffee beans at cherry stage after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2018/2019. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

The EC was higher in T1 (221 µS cm^{-1} g^{-1}) and lower in T5 (132 µS cm^{-1} g^{-1}) and the control (117 µS cm^{-1} g^{-1}). T2 was 24% lower than T1 (Figure 7B). Potassium leached (KL) more in treatments where the proportion of KCl was higher than K_2SO_4 (Figure 7B). T1, T2, and T3 had similar KL (~37 µg g^{-1}) while the other treatments were lower (~29 µg g^{-1}). There was no significant variation for the other variables, and the means were: 9.1% for TS, 1.03% for Caf, 46 u min^{-1} g^{-1} for PPO, 190 mL NaOH 100 g^{-1} of sample for TTA, and 5.0% for the content of Pol (Table 2).

Table 2. Variables analyzed in the coffee beans for the harvest of 2018/2019.

Treatments	Pol	TS	Caf	PPO	TTA
T1	4.9a	9.4a	1.05a	47.0a	188.6a
T2	5.1a	9.0a	1.03a	45.7a	189.7a
T3	4.8a	8.9a	1.02a	47.8a	193.1a
T4	5.0a	9.5a	1.03a	45.9a	194.9a
T5	5.1a	9.0a	1.03a	47.7a	191.3a
Control	5.2a	8.9a	1.02a	44.5a	187.1a
CV (%)	6.9	5.3	2.8	8.5	2.9
Mean	5.0	9.1	1.03	46.4	190.8

CV (%) = coefficient of variation; Pol = total phenolic compounds (%); TS = content of total sugars (%); Caf = content of caffeine (%); PPO = polyphenol oxidase activity (u min^{-1} g^{-1}); TTA = total tritable acidity (mL NaOH 0.1 N 100 g^{-1}). Means followed by the same letter in column do not differ according to Tukey's test ($p < 0.05$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

2.2.3. Harvest of 2019/2020

The highest grade was achieved in T5 (89 points) and the lowest was in the control (84) (Figure 8). However, T1 was not different from the control. T3 and T4 had similar scores (86 points) while T2 was similar to T3 and T4, but not different from T1. The other variables were not influenced by the application of the blends of K. The following means were found: 124 µS cm^{-1} g^{-1} for EC, 9.6% for TS, 1.02% for Caf, 54 u min^{-1} g^{-1} for PPO, 70.9 µg g^{-1} for KL, 195 mL NaOH 100 g^{-1} of sample for TTA, and 5.0% for Pol (Table 3).

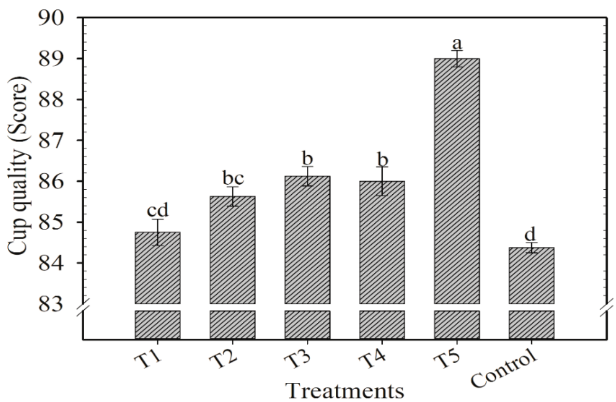

Figure 8. Scores of the cup quality of coffee beans at cherry stage after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2019/2020. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

Table 3. Variables analyzed in the coffee beans for the harvest of 2019/2020.

Treatments	Pol	TS	Caf	PPO	KL	TTA	EC
T1	5.1a	9.7a	1.05a	47.4a	72.7a	194.0a	119.4a
T2	5.2a	9.7a	1.09a	57.8a	69.9a	195.5a	129.7a
T3	5.1a	9.5a	1.08a	57.5a	62.8a	194.8a	119.7a
T4	5.1a	9.7a	1.06a	52.1a	75.2a	196.4a	126.7a
T5	5.2a	9.6a	1.03a	55.6a	80.2a	197.3a	134.7a
Control	5.0a	9.4a	1.03a	54.0a	64.2a	192.5a	118.4a
CV (%)	3.5	3.5	6.1	11.6	13.8	3.7	12
Mean	5.1	9.6	1.06	54.1	70.9	195	124

CV (%) = coefficient of variation; Pol = total phenolic compounds (%); TS = content of total sugars (%); Caf = content of caffeine (%); PPO = polyphenol oxidase activity (u min^{-1} g^{-1}); TTA = total tritable acidity (mL NaOH 0.1 N 100 g^{-1}). Means followed by the same letter in column do not differ according to Tukey's test ($p < 0.05$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O.

2.2.4. Principal Component Analysis (PCA) for the Agronomic Variables, Chemical Composition of the Beans, and Quality of the Coffee Beverage

The PCAs allow one to understand the behavior of the variables related to the chemical composition and quality of the coffee beverage in relation to the treatments, even if some of the variables were not statistically significant. Initially, we attempted to separate the treatments with ellipses in the PCAs calculated with all available data. However, we decided to add these PCAs in the Supplementary Material as we observed a lower percentage of the explained variance in comparison with the PCAs calculated without the agronomic data (those related to the soil). This happens when a set of variables with very different origins (soil, beverage quality, leaf composition) are used in PCA calculations (S5, S6, and S7).

Therefore, the treatments in the following PCAs represent the proportions of KCl and K_2SO_4 as described before. Thus, the closer they are, the greater the correlation between the variables that constitute these treatment groups. The agronomic variables stocks of K and Cl in the 0–20 and 20–80 cm layers, K and Cl contents in the leaves, yield, K and Cl removal in the beans and Cl content in the beans are supplementary variables; that is, they do not contribute to explaining the variability of the data. These illustrative variables are represented as dashed arrows, and they help to interpret the other data.

The PCA for the first harvest (2017/2018) indicates that the two components (Dim1 and Dim2) responded for 51.7% of the total variability of the data. The variances explained by these two variables were 38.2% and 18.9%, respectively (Figure S8). The variables K and Cl removal by the beans, K content in the leaves, yield, TS, Pol, and Caf were highly correlated. The control treatment was more related to these variables. In addition, these variables were negatively correlated to the stock of K in the 20–80 cm layer, stock of Cl in the 0–20 cm layer, and cup quality. The EC, KL, and TTA variables were highly correlated. The variables Cl content in the beans, stock of K in the 0–20 cm layer, and Cl content in the leaves were lowly correlated with the stock of Cl in the 20–80 cm layer and with the PPO activity. Furthermore, these variables were negatively correlated with the variables EC, KL, and TTA.

In the harvest of 2018/2019, the two PCA components explained 54.9% of the variability. The variances of each component were 39.6% and 15.3%, respectively (Figure 9). The cup quality was positively correlated with TS in the coffee beans and with yield. These variables are also correlated with T2. The KL variable was correlated with the stocks of Cl in both layers and with the Cl content in the leaves of the plants. T1 and T4 were close to these variables. Pol, Caf, and K and Cl in the beans were strongly correlated. Overall, the PCA shows that the quality of the coffee beverage is negatively correlated with the content of Cl in the leaves and beans.

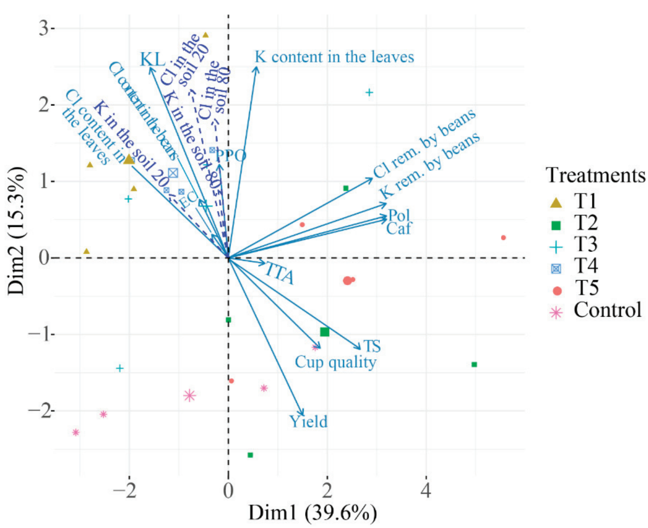

Figure 9. Principal component analysis for the harvest of 2018/2019. PPO = activity of the enzyme polyphenol oxidase; KL = K leaching; TTA = total titratable acidity; EC = electric conductivity; Pol = total phenolic compounds; Caf = content of caffeine; TS = content of total sugars; K in the soil 20 = stock of K in the 0–20 cm layer; K in the soil 80 = stock of K in the 20–80 cm layer; Cl in the soil 20 = stock of Cl in the 0–20 cm layer; Cl in the soil 80 = stock of Cl in the 20–80 cm layer, K rem. by beans: K removal by the beans, Cl rem. by beans: Cl removal by the beans. T1: 100% KCl, T2: 75% KCl + 25% K_2SO_4, T3: 50% KCl + 50% K_2SO_4, T4: 25% KCl + 75% K_2SO_4, T5: 100% K_2SO_4.

In the last harvest (2019/2020), the PCA explained 56.8% of the variability with the first component explaining 36.8%, and the second component explained 20% of the variance (Figure 10). The cup quality and PPO activity were closely related to T5. The stock of Cl in the 20–80 cm layer was strongly related to T1. KL and Cl contents in the leaves were also correlated with T1.

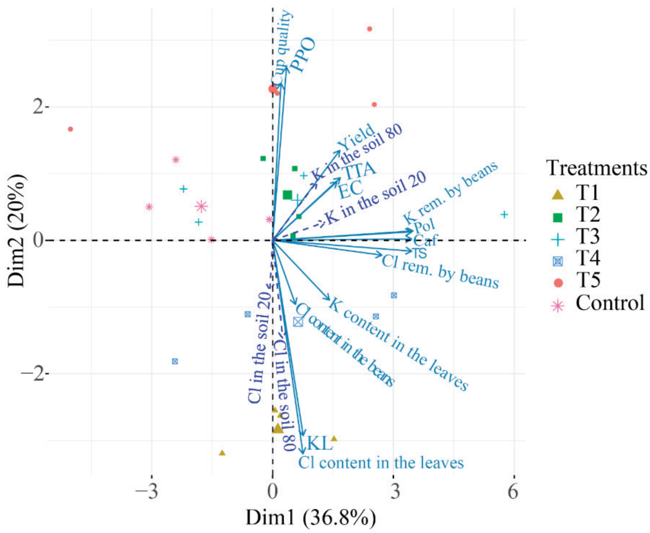

Figure 10. Principal component analysis for the harvest of 2019/2020. PPO = activity of the enzyme polyphenol oxidase; KL = K leaching; TTA = total titratable acidity; EC = electric conductivity; Pol = total phenolic compounds; Caf = content of caffeine; TS = content of total sugars; K in the soil 20 = stock of K in the 0–20 cm layer; K in the soil 80 = stock of K in the 20–80 cm layer; Cl in the soil 20 = stock of Cl in the 0–20 cm layer; Cl in the soil 80 = stock of Cl in the 20–80 cm layer, K rem. by beans: K removal by the beans, Cl rem. by beans: Cl removal by the beans. T1: 100% KCl, T2: 75% KCl + 25% K_2SO_4, T3: 50% KCl + 50% K_2SO_4, T4: 25% KCl + 75% K_2SO_4, T5: 100% K_2SO_4.

3. Discussion

Despite the long-time fertilization with KCl in the area, the initial K stocks in the soil were considered medium level [17]. For the initial amount of Cl, however, there is no method of extraction and no reference values to relate to the needs of coffee plants. Cl is a micronutrient that is required in low amounts by plants. Under field conditions, Cl deficiency is uncommon while the excess is frequently expressed.

The stocks of Cl reduced during the three years of study due to the leaching of the element to deeper layers in the soil. The Cl ion has low interaction with the soil solid phase [18]; thus, it is easily leachable [12].

There was a tendency to accumulate K in the leaves when plants received more KCl. KCl fertilizer is more soluble than K_2SO_4. Nonetheless, in all harvests, the foliar content of K remained adequate in the range of 19.7 to 31 g kg^{-1} [19,20] except for the low content in the control treatment in the last two harvests.

The Cl content in the leaves in all harvests was reduced from T1 to T5 and the control. The content of Cl usually found in plant tissues ranges from 2000 to 30,000 mg kg^{-1}, which is equivalent to the amount of macronutrients [21,22]. However, plants vary in their tolerance to Cl [23]. According to Marschner [22], plants sensitive to Cl show toxicity symptoms at concentrations higher than 3500 mg kg^{-1}. In tolerant plants, the symptoms appear when the concentration range from 20,000 to 30,000 mg kg^{-1}.

Under field conditions, toxicity symptoms caused by Cl excess are uncommon. Symptoms are characterized by the reduction of the width of the leaves, with possible curling, and the presence of wide necrosis with later leaf drying [11,12]. In this study, despite the high content found when KCl was applied (over 2500 mg kg^{-1}), plants did not show toxicity symptoms. However, it is important to emphasize the damages to the metabolism, growth, and yield that can occur even in concentrations below the toxicity threshold. In fact, in the harvest of 2019/2020, when the foliar content of Cl reached 4919 mg kg^{-1} in treatment T1, a lower yield was observed. An argument could be made for the higher availability of S in the treatments that received more K_2SO_4, but despite the source of K, all treatments received 2 t ha^{-1} of gypsum, which provided 340 kg ha^{-1} of S to the soil. The reduction in the yield is probably more related to the excess of Cl than the lack of S in the fertilization. Another aspect to be taken into account is that, under high concentrations of Cl in the soil, anion–anion competition may occur mainly with phosphate and nitrate ions. This is due to the inability of proteins to differentiate among nitrate, phosphate, and chlorine ions, leading to the absorption of the ion in higher concentration [12].

Conversely, the reduction in the yield is notable, even with no clear statistical separation, between the treatment that received only K_2SO_4 and the treatment fertilized solely with KCl. It is possible that such difference may be related to the solubility of the K_2SO_4, 80 g L^{-1} at 25 °C, which is considerably lower than the solubility of the KCl, 279 g L^{-1} [24]. This difference in the availability of K can compromise the yield in harvests of increased productivity as the harvest of 2019/2020. Another explanation is that the high solubility of KCl can benefit the absorption of cations, such as K, Ca, and Mg [25], increasing plant nutrition, even though it is for a short period. A third possibility is the excess of $SO_4{}^{2-}$, limiting the availability and absorption of $H_2PO_4{}^{-}$ by the plant, since, besides the K_2SO_4 application, gypsum was also applied.

The results suggest advantages in providing the two sources of K (25 to 75% of K_2SO_4) to increase yield. In the first two years of the experiment, when the yields were lower, the exportation of K by the beans was less intense and plant production was not limited by the sources of K once they were applied at the same dose.

The removal of K and Cl and the content of Cl in the beans were not different among the treatments since these elements remain in high concentration in the mucilage and the bean peels [26]. The exception is treatment T3 at the first harvest, but that might be related more to the history of the area than to the treatments.

3.1. Effects of the Application of KCl and K_2SO_4 Blends in the Chemical Composition of the Beans and in the Quality of the Coffee Beverage

There was a response in the KL in the first and second harvests. This variable is related to the integrity of the cell wall and membrane and, consequently, to the coffee beverage quality. When these structures are less intact, the cell has a higher tendency to lose cytoplasmatic contents as a reflection of the reduced cell organization [6,19,27]. The KL results for the first year of the evaluation showed the opposite effect to what would be expected, but this lower value observed for the T1 treatment is due to the influence of frequent fertilization with KCl before the evaluation. In the second year of evaluation, after the establishment of a new K dynamic in the soil and the reduction of Cl levels, less KL was found in treatments T4, T5, and the control.

Another piece of evidence for the reduction in the quality of the coffee beans and beverage with increasing doses of KCl is the high values of the EC observed in treatments T1, T2, and T3. As KL, CE also has a direct relationship with the integrity of the cell membrane [28,29].

Despite being considered indicatives of the quality of the beverage, these variables should not be decisive to vouch for the quality of the coffee [28]. In fact, the results of the cup quality in the last harvest suggest the same tendency observed for these variables. Notably, there was a response in the cup quality after the application of the treatments in the second harvest. As previously stated, in the first harvest, all response variables were very dependent on the previous fertilization in the area, thus the lack of response in the sensorial analysis.

However, from the second year of evaluation, some important facts should be emphasized about the K nutrition with the blends of fertilizers and the quality of the coffee beverage. Despite the lower scores for T1 and T2, the same behavior was observed for the control without K fertilization. This result suggests that only reducing the application of Cl via KCl is not enough to improve the quality of the beverage, but also maintaining adequate levels of K is essential to produce a high-quality coffee [6,15].

In the last harvest, treatment T5 achieved the highest score (89 points) in the sensorial analysis. T3 and T4, however, reached a few points less (86 points) than T5. Considering the higher cost of K_2SO_4 in relation to KCl, the choice for the composition of the K fertilizer should consider the economic cost that this difference of 3 points in the cup quality might return. Another important consideration is that there was a tendency for higher yield in T3 and T4 treatments despite the lack of statistical differences among the treatments. The difference between both treatments in relation to treatment T5 yielded more than five sacks of 60 kg of coffee beans, suggesting that yield should also be considered when choosing the best K fertilizer composition.

3.2. Principal Component Analyses for the Agronomic Variables and the Quality of the Coffee

The PCA results suggest that studies on how the fertilization of coffee plants affects the quality of the coffee beverage should be carried out for a long duration.

Overall, there were increased effects of the treatments after the second year of evaluation, probably due to the previous fertilizations with KCl, which is the most used source of K in Brazil [15]. However, some points should be considered in relation to the first harvest, such as the correlation among the variable Cl content in the beans, K content in the leaves, and yield showing the importance of the K fertilization in coffee plants. Nonetheless, the negative correlation of these variables with the cup quality suggests an unfavorable effect of one of these variables on the quality of the beverage, most probably the Cl content in the beans.

The correlation between EC and KL can be explained by the direct relationship shared by these two variables since they both indicate damages to the cell integrity of the beans [28–30]. The PCA confirms these results. These damages can lead to the loss of compounds related to the quality of the beans and the cup quality; therefore, lower EC and KL indicate lower coffee quality [28,31]. This lower bean quality is confirmed by the high negative correlation between PPO activity and the variables EC, PL, and TTA, showing that higher values of EC, KL, and TTA are associated with low PPO activity. Several studies found a positive correlation between the PPO activity and the sensorial quality of the coffee [32,33]. Thus, it is possible to conclude that there is a reduction in the PPO activity and the quality of the beverage as EC, KL, and TTA increase. In fact, damages to the cell membrane lead to the loss of selective permeability, facilitating the reaction of PPO with the phenolic compounds (the specific substrate of this enzyme). This reaction produces quinones that inhibit the activity of PPO [16,32].

Noteworthy, the high positive correlation between cup quality and the content of TS in the beans indicates a direct relationship where the increase in TS also increases the cup quality. Treatment T2 was close to this correlation, confirming the results for cup quality and indicating that increases in the K_2SO_4 proportion tend to increase cup quality. These results confirm the study of Silva et al. [16], which also involved doses and sources of K, and they add information about the sensorial quality of the coffee beverage. These findings provide evidence for the increase in the content of TS and better scores on the cup quality test as the proportion of K_2SO_4 in the blend also increases.

The reduction in the quality of the coffee beans is observed in the high correlation among KL, the stock of Cl in the soil, and the Cl content in the leaves. When the values of the variables related to Cl increase, K also increases, and the quality of the beans and the beverage reduces.

In the last harvest (2019/2020), a direct effect of the Cl in the variables related to the quality of the beverage is notable. Despite the fact that the PPO activity did not show differences for the treatments in both harvests, this variable behavior within the PCA is enough to indicate the direct relationship of this enzyme with the quality of the coffee beverage.

In addition, the high negative correlations of cup quality and PPO in relation to the variables related to Cl (stocks in the soil and content in the leaves) confirm the negative influence of the Cl in the beverage. In addition, treatment T5, which received only K_2SO_4, is closely related to cup quality and PPO activity.

Previous reports state that Cl increases the water content of the coffee fruits with consequent microbial fermentation [13,14]. We believe this explanation does not relate to this study since the beans were collected manually at the stage of cherry and benefited under controlled conditions, unlikely leading to an undesirable fermentation.

Although we did not perform physiological or morphological analyses of the coffee beans, the results allow us to infer that there is an effect of the Cl in the beans and that it might be related to the loss of quality of the beverage. The rationale is that the Cl can inhibit the activity of the PPO enzyme when reacting with the copper activator, thus reducing the enzymatic activity when KCl is applied [34].

In conclusion, this study showed that the blends of K fertilizers responded positively to the quality of the coffee beverage when the proportion of K_2SO_4 relative to KCl was increased. There was a tendency for higher KL, EC, and TTA with the increase of the KCl proportion, which might lead to damages in the cell membrane caused by the Cl and the consequent reduction of the PPO activity and quality of the beverage. However, the decision to use a determined blend of K should consider the improvement of the beverage and the economic return to the farm. Moreover, it should also consider the yield. For example, in this study, the blend that provided the best quality for the coffee beverage was not always the same responsible for the highest yield. And finally, it should also consider the economic costs of fertilization with K_2SO_4, which is more expensive than KCl. Considering these aspects, a management strategy could be the separation of the farm into plots based on the tendency to produce better quality coffees in previous years. In this case, each plot would receive a determined blend of K, and the highest proportions of K_2SO_4 should be applied to the plots with a tendency to produce higher quality coffees while the higher proportion of KCl would fertilize plots of low-quality coffee. Finally, any investigation with similar objectives to this work should perform a broader study, especially covering the regions of greater coffee production, where each plot/farm or region would receive potassium fertilization depending on the tendency for better or worse quality of the coffee beverage in the cup.

4. Materials and Methods

4.1. Experimental Area Characterization

The experiment was performed through three consecutive years (harvests of 2017/2018, 2018/2019, and 2019/2020) in a commercial production system of coffee located in the municipality of Santo Antônio do Amparo-MG, Brazil (20°53′26.04″ S and 44°52′04.14″ W and mean altitude of 1100 m). The plantation of Coffea arabica L., cultivar Catuaí Vermelho IAC 99, initiated in 2012 and spaced at 3.40 m × 0.65 m, is planted on a clayey Dystrophic Red Latosol-Latossolo Vermelho distrófico (Oxysol) [35].

Before the experiment, soil samples were collected for chemical attributes and texture analyses (Table 4). Samples from the 0–80 cm layer of soil were collected to assess K and Cl stocks. Undisturbed soil samples were taken to assess bulk density (BD). For depths over 5 cm, multiple samples were taken followed by the weighted average of the BD values. After determining K and Cl concentrations (mg kg^{-1}), the values were multiplied by the BD to transform them into kg ha^{-1}.

Table 4. Soil analyses results on September 2017.

Depth	pH $CaCl_2$	K^+	P	Ca^{2+}	Mg^{2+}	Al^{3+}	H + Al	BS	ECEC	CEC	V	m
cm	-	mg dm^{-3}		cmol$_c$ dm^{-3}							%	
0–10	5.2	96	7.9	1.4	0.7	0.3	8.7	2.5	2.8	11.2	22.2	11.9
10–20	5.2	87	9.2	1.5	1.1	0.1	6.7	3.0	3.1	9.7	30.8	5.6
20–40	5.1	69	7.1	1.6	1.0	0.1	6.5	3.0	3.1	9.5	31.5	5.0
Depth	**OM**	**P(rem)**	**Zn^{2+}**	**Fe^{2+}**	**Mn^{2+}**	**Cu^{2+}**	**B**	**S**	**Sand**	**Silt**	**Clay**	
cm	dag kg^{-1}	mg L^{-1}	mg dm^{-3}						%			
0–10	3.8	17.2	1.8	57.6	8.4	2.1	0.2	180	22	14	64	
10–20	3.7	15.9	2.2	52.4	7.7	2.1	0.3	90	22	16	62	
20–40	3.6	14.6	1.3	39.3	4.2	2.0	0.3	48	22	18	60	

P, K^+, Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}—Mehlich extractor. Ca^{2+}, Mg^{2+}, Al^{3+}—1 mol L^{-1} KCl extractor. H^+ + Al^{3+}—SMP extractor. B—hot water extractor. S—monocalcium phosphate in acetic acid extractor. BS = exchangeable bases sum. ECEC = effective cation exchange capacity. CEC = cation exchange capacity at pH 7.0. V = base saturation. m = aluminum saturation. P-rem = remaining phosphorus. OM = organic matter (oxidation with $Na_2Cr_2O_7$ 0.57 mol L^{-1} + H_2SO_4 5 mol L^{-1}).

4.2. Experimental Design

The experimental design was in randomized blocks, with four blocks disposed at 90 degrees with the slope of the area. The treatments were composed of blends of KCl and K_2SO_4 (both in terms of K_2O) as follows: T1—100% as KCl; T2—75% as KCl + 25% as K_2SO_4; T3—50% as KCl + 50% as K_2SO_4; T4 –25% as KCl + 75% as K_2SO_4; T5: 100% as K_2SO_4; and a control without K_2O application. Each plot was composed of three planting lines with 16 plants, and the 10 central plants were considered a useful area (Figure 11).

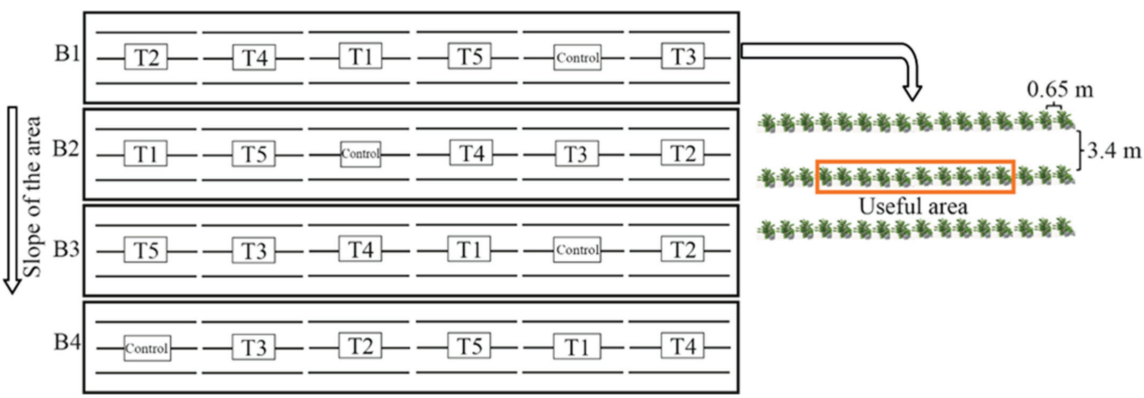

Figure 11. Schematic representation of the experimental design, number of plants in each plot, and the useful area used to collect the data.

4.3. Experiment Conducting

4.3.1. Liming, Fertilization, and Gypsum Application

After the coffee harvest of each studied year, soil samples from the 0–10 cm, 0–20 cm, and 20–40 cm layers were collected to evaluate the needs for liming, fertilization, and gypsum application, respectively [17]. Liming was applied at 1.0 t ha^{-1}, 1.2 t ha^{-1}, and 1.5 t ha^{-1} on the first, second, and third harvest years, respectively. Gypsum was applied at 1.1 t ha^{-1} and 2.0 t ha^{-1} in the second and third years, respectively. Both dolomite lime and gypsum were applied underneath the projection of the tree canopies. P was applied at 120, 90, and 90 kg ha^{-1} of P_2O_5 as triple superphosphate on each consecutive year. N was applied at 350, 350, and 400 kg ha^{-1} of N as ammonium nitrate on each consecutive year, divided into three applications.

4.3.2. Potassium Fertilization

Before the experiment, the saline index of each blend of K fertilizer was determined by comparing a 10 g L^{-1} sodium nitrate solution with solutions prepared with the blends of KCl e K_2SO_4 at the same concentration (Jackson 1958). The electric conductivity of the solutions and the saline index were calculated according to the equation: SI = [((ECa))⁄((ECb))] × 100, where SI is the saline index, ECa is the electric conductivity of the sample, and ECb is the electric conductivity of the sodium nitrate solution. The SI found were 142, 130, 121, 112, and 104%, for T1, T2, T3, T4, and T5, respectively.

The maintenance fertilization was done according to Guimarães et al. [36] using the abovementioned blends. The doses of K_2O applied were 150, 200, and 300 kg ha^{-1} for the respective agricultural years of 2017/2018, 2018/2019, and 2019/2020. All K fertilizations were divided into three applications.

4.3.3. Agronomic Variables Assessed on the Three Harvests

- K and Cl content in the leaves

The third and fourth pair of leaves on both sides of the plants were collected from the useful area 20 days after the second application of the cover fertilization. The leaves were washed in deionized water, dried at 65 °C, and grounded in a Willey mill. The plant material was digested in a solution of nitric-perchloric acid (4 parts of nitric acid to 1 part of perchloric acid), and K was determined with inductively coupled plasma (ICP). To determine Cl, 1 g of grounded material was added to 50 mL of ultrapure water under agitation for 15 min [37]. After filtering the extract, the content of Cl (mg kg^{-1}) was determined with a selective electrode (Hanna®, model HI4107) coupled to a Hanna® device, model HI2221. The determination curve was built using the concentrations of 2, 20, 200, and 1000 mg L^{-1} of Cl.

- Yield

The harvests were done when more than 70% of the fruits were mature. For the chemical analyses, 4 L of beans at the cherry stage were collected two days before each harvest. Fruits were peeled with an electric peeler (Pinhalense®, model DPM-02) and submerged for 24 h to remove the mucilage. After removing the peels and the rotten beans, samples were air-dried until a 10.8% to 11.2% moisture level.

The yield was determined by harvesting all fruits in the useful area. After the harvest, 5 L of a mix of fruits in every stage of maturation were air-dried under sunlight for one day. When the samples reached around 12% of moisture, beans were peeled and weighted. The moisture level was then adjusted to 12%, which is considered adequate for commercialization. To estimate yield, the weight of the beans in the useful area was projected to the number of plants in one hectare (4524 plants).

- K and Cl content and removal in the beans

K and Cl contents in the beans were determined at the cherry stage after air-drying (65 °C, until constant weight) and grounding the beans in a Willey mill. K content was determined after nitric-perchloric digestion with measures done by ICP. Cl content followed the same procedures to quantify Cl in the leaves. The amounts of these elements removed from the soil were obtained by multiplying their content in the beans by the yield on each treatment.

- Stocks of Cl in the soil

Stocks of Cl in the 0–20 and 20–80 cm layers of soil were checked during the experiment. Six soil samples were taken from the soil underneath the projection of the tree canopies (three from each side of the parcel). Extraction and determination of Cl followed the same procedure described for leaf Cl content, but with the proportion of 10 g of soil to 50 mL of ultrapure water. The stocks were determined by multiplying the element concentration by the mass of soil in each layer.

The analytical standard Tomato leaves (NIST 1573A), with 0.66% of Cl, was used in both soil and plant material analysis. The mean recovery of Cl was higher than 92%, assuring that the extraction and determination used for Cl were effective for both soil and plant material.

- Chemical analysis of the beans and coffee sensorial analysis

After benefiting the coffee samples, the beans were stored in paper bags in a cold chamber until the chemical and sensorial analyses. The chemical analyses were performed at the Laboratory of Coffee Quality Analysis in the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG). Potassium leaching (KL, in μg g^{-1}) was determined after 5 h of soaking [29], and electric conductivity (EC, in μS cm^{-1} g^{-1}) was determined according to Loeffler et al. [38]. The total titratable acidity (TTA, m mL NaOH 0.1 N 100 g^{-1}) was done according to Carvalho et al. [32] in the adaptation of the methodology from the Association of Official Analytical Chemists [39]. The content of total sugars (TS, in %) followed the anthrone method [40]. The activity of the polyphenol oxidase enzyme (PPO, in u min^{-1} g^{-1}) was determined according to Carvalho et al. [32]. Total phenolic compounds (Pol, in %) were extracted according to Goldstein and Swain [41] and determined by the Folin-Denis method, described by AOAC [39]. Caffeine content (Caf, in %) was determined by spectrophotometry at 273 nm [42]. The coffee beans were frozen in liquid nitrogen and grounded in an IKA mill for the analyses, except for the KL and EC determinations.

The sensorial analysis (cup quality) was performed at the Laboratory of Agricultural Products Processing in the Universidade Federal de Lavras following the Specialty Coffee Association of America (SCAA) protocol. Three professionals with skills to differentiate fragrances, characteristics, and flavors participated in the cup test. The evaluation was based on scores given to the following attributes: fragrance/aroma, uniformity, clean cup, sweetness, flavor, acidity, body, aftertaste, balance, defects, and overall. The coffees were classified as the SCAA [43] according to their final scores (Table 5).

Table 5. Coffee beverage classification according to the cup quality.

Final Score	Special Description	Classification
95–100	Outstanding	Super premium specialty
94–90	Excepcional	Premium specialty
85–89	Excellent	Specialty
84–80	Very good	Specialty
75–79	Good	Good quality—normal
74–70	Weak	Medium quality

Source: Specialty Coffee Association of America (SCAA) (2009).

- Statistical analyses

After model validation and analysis of variance indicating differences among treatments ($p < 0.05$), the response variables were submitted to Tukey's test ($p < 0.05$) on the R 3.3.1 environment [44]. Principal component analyses (PCA) were performed to correlate the agronomic variables with the coffee beverage variables and yield. In the PCA, two components (Dim1 and Dim2) were used to represent the total data variability. The package Facto MineR (version 1.42) was used in the R software.

5. Conclusions

The activity of the polyphenol oxidase enzyme and the cup quality indicate that the ion Cl- reduces the quality of the coffee beverage. The increased application of the Cl- ion increases KL, EC, and TTA, indicators of the loss of coffee quality. K content in the leaves was not influenced by the application of blends of K fertilizer while Cl content increased linearly with KCl applied. The application of KCl and K_2SO_4 blends influenced coffee yield and the optimum proportion was 25% of KCl and 75% of K_2SO_4. The highest score in the cup quality test was observed with 100% K_2SO_4. However, other blends showed close scores. The decision for the fertilizer should consider the cost of the K source. KL and EC can indirectly show that the Cl can damage the coffee beans and reduce the selective permeability of the cell membrane, with possible negative consequences to the coffee beverage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants12040885/s1, Table S1. Variables analyzed in the coffee beans for the harvest of 2017/2018; Figure S1. Stocks of Cl in the 0–20 and 20–80 cm layers after application of KCl and K_2SO_4 blends as cover fertilization on coffee plants. Harvest of 2017/2018. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O; Figure S2. K and Cl content in the leaves of coffee plants, 20 days after application of the second cover fertilization parcel. Harvest of 2017/2018. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O; Figure S3. Yield of coffee plants and Cl content in the beans at cherry stage (A) and K and Cl removal by the beans (B) after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2017/2018. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O; Figure S4. Potassium leaching in coffee beans at stage of cherry after application of blends of KCl e K_2SO_4 as cover fertilization. Harvest of 2017/2018. Means followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$). Vertical bars indicate the standard error of the mean ($n = 4$). T1: 100% KCl; T2: 75% KCl + 25% K_2SO_4; T3: 50% KCl + 50% K_2SO_4; T4: 25% KCl + 75% K_2SO_4; T5: 100% K_2SO_4; control did not receive K_2O; Figure S5. Principal component analysis for the harvest of 2017/2018. PPO = activity of the enzyme polyphenol oxidase; KL = K leaching; TTA = total titratable acidity; EC = electric conductivity; Pol = total phenolic compounds; Caf = content of caffeine; TS = content of total sugars; K in the soil 20 = stock of K in the 0-20 cm layer; K in the soil 80 = stock of K in the 20-80 cm layer; Cl in the soil 20 = stock of Cl in the 0-20 cm layer; Cl in the soil 80 = stock of Cl in the 20-80 cm layer, K rem. by beans: K removal by the beans, Cl rem. by beans: Cl removal by the beans. T1: 100% KCl, T2: 75% KCl + 25% K_2SO_4, T3: 50% KCl + 50% K_2SO_4, T4: 25% KCl + 75% K_2SO_4, T5: 100% K_2SO_4; control did not receive K_2O. Figure S6. Principal component analysis for the harvest of 2018/2019 considering agronomic data. Figure S7. Principal component analysis for the harvest of 2019/2020 considering agronomic data. Figure S8. Principal component analysis for the harvest of 2017/2018.

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