

Article

The Role of Green Manure in Wheat Production on Low-Fertility Soils in Northwestern Romania

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Abstract

Green manuring may improve crop performance on acidic, low-fertility soils, although its effects depend on the incorporated species and mineral fertilization regime. This study evaluated the effects of five green manure crops and mineral fertilization on winter wheat during the 2021/2022 growing season at the Agricultural Research and Development Station Livada, northwestern Romania. A two-factor split-plot field experiment was conducted at a single location during one growing season (2021/2022) using three replicate blocks. Six main-plot treatments—wheat, triticale, pea, narrow-leafed lupine, oilseed rape, and a non-sown control with spontaneous vegetation—were combined with two subplot regimes: with and without combined N–P mineral fertilization. The green manure × mineral fertilization interaction was significant for grain yield ($p = 0.008$). Under mineral fertilization, pea produced the highest numerical grain yield ($8018 \pm 116 \text{ kg ha}^{-1}$; mean $\pm$ SE, $n = 3$), compared with $6730 \pm 244 \text{ kg ha}^{-1}$ in the corresponding fertilized control. Without mineral fertilization, pea and lupine produced 5427 ± 187 and $5009 \pm 237 \text{ kg ha}^{-1}$, respectively, compared with $3419 \pm 108 \text{ kg ha}^{-1}$ in the corresponding non-fertilized control. Wheat and triticale green manures were associated with lower subsequent wheat yields than their corresponding controls under both fertilization regimes. Under the conditions of this single-site, single-season experiment, pea and narrow-leafed lupine were associated with favorable grain-yield responses, particularly relative to the corresponding non-sown controls. Further multi-year and multi-location experiments are required to determine the stability of these responses under contrasting soil and climatic conditions.



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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops and plays a major role in global food security. However, maintaining stable wheat production increasingly requires agricultural practices that improve nutrient availability while limiting soil degradation and excessive dependence on mineral fertilizers. Concerns regarding soil degradation, nutrient losses, and the long-term maintenance of crop productivity have

increased interest in integrated soil fertility management practices, including the use of green manure crops [1].

In this broader context, green manuring is being investigated as a complementary practice within cereal-based cropping systems.

Green manure crops are cultivated primarily to produce biomass that is subsequently incorporated into the soil rather than harvested as a commercial product. Previous studies indicate that, following incorporation and decomposition, green manure biomass may return organic matter and nutrients to the soil and may influence several soil processes and properties [1]. However, the responses reported in the literature vary according to the green manure species, the quantity and composition of the incorporated biomass, the developmental stage at incorporation, soil and climatic conditions, and the management of the subsequent crop [2,3].

Pea (*Pisum sativum* L.) and narrow-leaved lupine (*Lupinus angustifolius* L.) were selected in the present study as representatives of leguminous green manures. According to previous research, legume residues frequently have a lower carbon-to-nitrogen ratio than cereal residues and may therefore undergo more rapid decomposition and nitrogen mineralization [4,5]. These characteristics may support the growth of a subsequent cereal crop, although their effects depend on the quantity and composition of the incorporated biomass and on the environmental and management conditions [4,6]. In the present experiment, biological nitrogen fixation, residue nitrogen concentration, dry biomass, and decomposition dynamics were not measured; consequently, these mechanisms are considered potential explanations derived from the literature rather than processes demonstrated directly by the study.

Non-leguminous species were selected to represent green manures with characteristics distinct from those of legumes. Wheat and triticale ($\times$ *Triticosecale* Wittm.) were included as representatives of cereal green manures because of their rapid establishment and capacity to produce substantial vegetative cover, which may influence weed establishment [1,7]. However, cereal residues commonly have a higher carbon-to-nitrogen ratio than legume residues and may temporarily immobilize soil nitrogen during decomposition [4,5]. The inclusion of wheat as a green manure before winter wheat also allowed the assessment of an atypical wheat–wheat sequence. Previous research indicates that continuous wheat cultivation may maintain host continuity and increase the risk of certain wheat diseases [2]. Oilseed rape (*Brassica napus* L.) was selected as a representative of the Brassicaceae, providing a non-leguminous broadleaf treatment with growth and residue characteristics different from those of cereals and legumes [4]. Thus, the selected species represented three contrasting botanical and functional groups that could produce different responses in the subsequent winter wheat crop. Residue composition, nitrogen immobilization, allelopathic compounds, and pathogen incidence were not measured directly; therefore, these mechanisms were not treated as experimentally demonstrated effects. Evaluating green manure crops is particularly relevant under acidic, low-fertility soil conditions, where crop responses may differ according to the incorporated species and the mineral fertilization regime. Low soil pH can restrict nutrient availability and root development and may reduce the effectiveness of applied fertilizers [8]. At the experimental site in northwestern Romania, the surface soil had a pH of 5.19, a humus content of 1.82%, total nitrogen of 0.168%, plant-available phosphorus of 13.6 mg kg^{−1}, plant-available potassium of 100 mg kg^{−1}, and a base saturation of 53.5%. Taken together, the low pH, humus content of 1.82%, and plant-available phosphorus content of 13.6 mg kg^{−1} indicate that soil acidity and restricted nutrient availability could constrain crop performance at the experimental site. The plant-available potassium content was reported separately and was not considered deficient.

Although green manures have been investigated in various cropping systems, their effects are strongly influenced by species, soil conditions, climate, and the fertilization regime applied to the subsequent crop [9–11]. Evidence from northwestern Romania remains limited regarding the direct comparison of leguminous, cereal, and brassica green manures under paired mineral-fertilized and non-mineral-fertilized conditions. Previous research conducted in the region has examined the effects of green manure on the fertility and acidity of Albic Luvisols [9], whereas studies performed in other environments have evaluated the responses of winter wheat to green manure and mineral fertilization [10,11]. However, the comparative response of winter wheat yield and physical grain characteristics to these three botanical groups under the acidic, low-fertility conditions of northwestern Romania remains insufficiently documented. The present study addresses this regional evidence gap through a single-site, single-season field comparison rather than claiming broad or universal applicability. Accordingly, this single-site, single-season study aimed to quantify the response of winter wheat cv. Glosa to five green manure species—wheat, triticale, pea, narrow-leaved lupine, and oilseed rape—and a non-sown control with spontaneous vegetation, under two mineral fertilization regimes: with and without combined N–P fertilization. The specific objectives were to determine the effects of the treatments on: (i) wheat grain yield; (ii) grain moisture content; (iii) thousand-kernel weight; (iv) hectoliter weight; and (v) weed density before herbicide application. The following hypotheses were tested: (H1) the response of winter wheat would differ among the green manure treatments; (H2) the effect of the green manure treatment would depend on the combined N–P mineral fertilization regime; and (H3) leguminous green manure treatments would be associated with higher subsequent wheat yields than cereal green manure treatments. These hypotheses concerned the measured agronomic responses and did not presume changes in biological nitrogen fixation, nutrient availability, residue decomposition, soil properties, or fertilizer-use efficiency, which were not measured directly.

2. Materials and Methods

2.1. Experimental Site, Initial Soil Conditions, and Field History

The field experiment was conducted during the 2021/2022 growing season at the Agricultural Research and Development Station Livada (SCDA Livada), Satu Mare County, northwestern Romania. Meteorological data were obtained from the weather station located at SCDA Livada.

The experimental soil was classified as a stagno-gleyed preluvo-soil according to the Romanian System of Soil Taxonomy and as a Stagnic Luvisol according to the World Reference Base for Soil Resources (WRB). Before trial establishment, soil samples were collected from the 0–15 cm layer. The topsoil had a pH of 5.19, measured in water, a humus content of 1.82%, a total nitrogen content of 0.168%, plant-available phosphorus and potassium contents of 13.6 and 100 mg kg^{−1}, respectively, a clay content of 20.9%, and a base saturation of 53.5%. The soil was acidic and had a low humus content and limited plant-available phosphorus, whereas plant-available potassium was not considered deficient. These initial properties provide the specific edaphic context in which the treatment responses were evaluated.

The experimental field had received no liming amendments or organic fertilizers since 1990 and had been maintained under a crop rotation comprising wheat, maize, sunflower, and oilseed rape. The crop preceding the green manure treatments was winter wheat. Before sowing, the preceding wheat crop received 150 kg ha^{−1} of 18–46–0 compound fertilizer, followed by 400 kg ha^{−1} of nitrocalcar in spring. No subsoiling or other deep soil-loosening operations had been performed on the experimental field. This management history was

reported because residual effects of the preceding crop and previous fertilization could have influenced the initial soil conditions and the responses of the subsequent treatments.

2.2. Experimental Design

The effects of green manure treatment and combined N–P mineral fertilization on the subsequent winter wheat crop were evaluated using a two-factor split-plot field design with three replicate blocks. The green manure treatment represented the main-plot factor (Factor A) and comprised six levels: wheat (*Triticum aestivum* L., cv. Glosa), triticale ($\times$ *Triticosecale* Wittmack, cv. Negoiu), pea (*Pisum sativum* L., cv. Magistra Liv), narrow-leaved lupine (*Lupinus angustifolius* L., cv. Medi), oilseed rape (*Brassica napus* L., cv. Umberto), and a non-sown control with spontaneous vegetation. No green manure crop was sown in the control treatment; however, the spontaneous vegetation that developed in these plots was incorporated into the soil.

Combined N–P mineral fertilization represented the subplot factor (Factor B) and comprised two levels: with mineral fertilizer and without mineral fertilizer. Within each replicate block, the six green manure treatments were arranged in the order shown in Figure 1. Each main plot was divided into two equal subplots, one without mineral fertilizer and one receiving combined N–P mineral fertilization. Because an independent random allocation of the treatments was not documented, the manuscript does not describe the treatment arrangement as randomized.

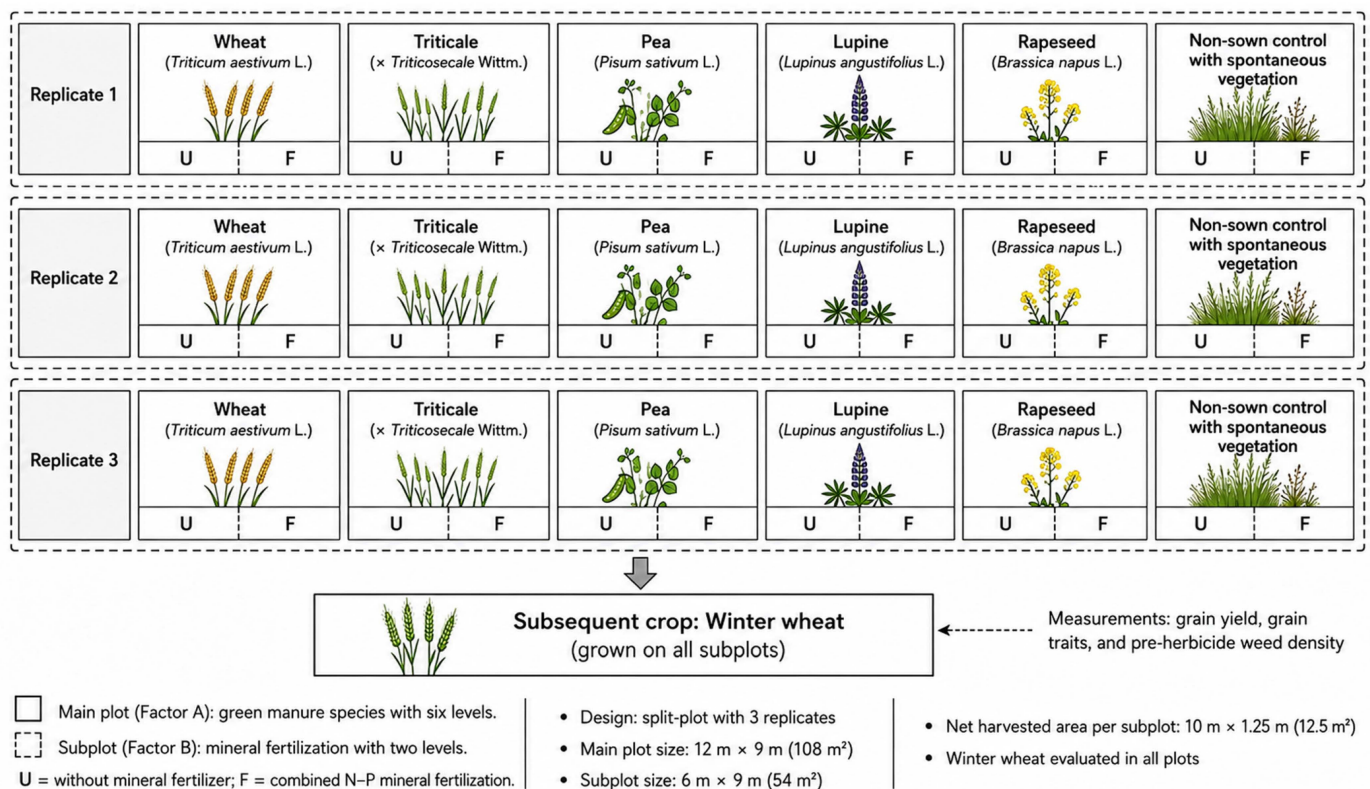


Figure 1. Schematic representation of the two-factor split-plot field structure and treatment arrangement used at SCDA Livada during the 2021/2022 growing season. Factor A, applied to the main plots, comprised wheat, triticale, pea, narrow-leaved lupine, oilseed rape, and a non-sown control with spontaneous vegetation. Factor B, applied to the subplots, comprised two combined N–P mineral fertilization regimes: with and without mineral fertilizer. U, without mineral fertilizer; F, with combined N–P mineral fertilization. Each treatment combination was represented once in each of the three replicate blocks.

Each main plot measured $12\text{ m} \times 9\text{ m}$, corresponding to a total area of 108 m^2 , and was divided into two subplots measuring $12\text{ m} \times 4.5\text{ m}$, corresponding to an area of 54 m^2 each. The net harvested area was 12.5 m^2 ($10\text{ m} \times 1.25\text{ m}$) within each subplot. Measurements and harvesting were conducted within this internal area to reduce potential edge effects. No separate buffer-strip dimensions were recorded; therefore, no undocumented buffer distances are reported.

A schematic representation of the split-plot experimental structure and treatment arrangement is provided in Figure 1.

2.3. Establishment, Biomass Sampling, and Incorporation of the Green Manure Crops

The green manure crops were sown on 23 July 2021, immediately after the harvest of the preceding wheat crop. Wheat and triticale were sown at a rate of 250 kg ha^{-1} with a row spacing of 12.5 cm . Pea, narrow-leaved lupine, and oilseed rape were sown at rates of 120, 200, and 4 kg ha^{-1} , respectively, with a row spacing of 25 cm .

The wheat and triticale seeds were supplied by the National Agricultural Research and Development Institute Fundulea (INCDIA Fundulea), the pea and lupine seeds by SCDA Livada, and the oilseed rape seeds by KWS. The cultivars used were Glosa for wheat, Negoiu for triticale, Magistra Liv for pea, Medi for narrow-leaved lupine, and Umberto for oilseed rape.

The green manure crops were incorporated into the soil on 14 October 2021. At incorporation, wheat and triticale were at the end of tillering to the beginning of stem elongation (BBCH 29–30), pea and lupine were at inflorescence emergence to the beginning of flowering (BBCH 55–60), and oilseed rape was at the side-shoot development to the beginning of stem elongation stage (BBCH 25–30).

Fresh plant biomass was assessed one day before incorporation using five 1 m^2 sampling areas for each green manure species. The sampling areas were positioned at 6 m intervals across the corresponding treatment plots. Within each sampling area, the cultivated green manure plants were removed with a spade to a depth of approximately $20\text{--}25\text{ cm}$, including both aboveground parts and roots. Soil particles were removed from the roots before weighing, and only the biomass of the cultivated green manure species was included; spontaneous vegetation occurring within the sampled area was excluded. Fresh biomass expressed in kg m^{-2} was converted to t ha^{-1} using the conversion factor $1\text{ kg m}^{-2} = 10\text{ t ha}^{-1}$. The resulting mean fresh biomass values were 22.5 t ha^{-1} for wheat, 21.9 t ha^{-1} for triticale, 28.3 t ha^{-1} for pea, 26.0 t ha^{-1} for narrow-leaved lupine, and 25.7 t ha^{-1} for oilseed rape.

Biomass was weighed only in the fresh state. Dry biomass, dry matter content, residue carbon and nitrogen concentrations, the C:N ratio, and the quantity of nitrogen incorporated with the biomass were not determined. The reported fresh biomass values are therefore presented descriptively and should not be interpreted as direct estimates of dry organic matter or nutrient inputs. No statistical comparison of biomass among species was performed. The green manure crops were not harvested for commercial use.

The standing vegetation was chopped and incorporated superficially through two passes with a GD 3.5 disk harrow and was subsequently incorporated into the soil by moldboard plowing with a PP 3-35M plow at a depth of $25\text{--}27\text{ cm}$ (Figure 2). Disk harrowing and moldboard plowing were performed on the same day and were applied uniformly to all treatments, including the non-sown control with spontaneous vegetation.

No green manure crop was sown in the non-sown control with spontaneous vegetation. The vegetation observed in this treatment consisted mainly of volunteer wheat and monocotyledonous weeds, including *Eriochloa villosa*, *Echinochloa crus-galli*, and *Setaria viridis*, together with a smaller proportion of dicotyledonous species, including *Chenopodium album*,

Veronica arvensis, and *Polygala amara*. This spontaneous vegetation was incorporated using the same disk-harrowing and moldboard-plowing operations applied to the sown green manure treatments. Spontaneous vegetation was also observed within the sown treatments, although its density and biomass were not determined separately.

The fresh and dry biomass of the spontaneous vegetation in the non-sown control was not quantified separately. Consequently, the control treatment cannot be considered bare fallow, and the observed differences cannot be attributed exclusively to the identity of the sown green manure species independently of differences in total incorporated biomass. This limitation was considered when interpreting the treatment effects.



Figure 2. Disk harrowing of the narrow-leaved lupine green manure crop before soil incorporation at SCDA Livada.

2.4. Winter Wheat Establishment and Crop Management

Following the incorporation of the green manure treatments by moldboard plowing, the soil was left for 7–8 days. The field was then disk-harrowed twice, followed by one pass with a spike-tooth harrow and one pass with a Multiflex seedbed cultivator. These seedbed-preparation operations were performed uniformly across all treatments. Winter wheat (*Triticum aestivum* L.) cv. Glosa was subsequently sown in all subplots. Glosa is a Romanian winter wheat cultivar developed by the National Agricultural Research and Development Institute Fundulea (INCDIA Fundulea). The seed used in the experiment was produced by SCDA Livada, belonged to the basic seed category, and had a germination capacity of 98.2%.

Winter wheat was established at a density of 550 viable seeds m^{-2} , corresponding to a seeding rate of approximately 250 kg ha^{-1} . The row spacing was 12.5 cm, and the sowing depth was 3–4 cm. Before sowing, the seed was treated with AmiralProffy at a rate of 0.5 L t^{-1} of seed. The product contained tebuconazole at a concentration of 60 g L^{-1} .

Weed control was performed uniformly in all winter wheat subplots at BBCH 29–30 using Sekator Progress OD at a rate of 0.15 L ha^{-1} . The herbicide contained amidosulfuron (100 g L^{-1}), iodosulfuron-methyl-sodium (25 g L^{-1}), and mefenpyr-diethyl (250 g L^{-1}) as a safener. Weed density was assessed before this uniform herbicide application, as described in Section 2.8. No treatment-specific differences in weed control or seedbed preparation were introduced. Except for the combined N–P mineral fertilization treatment defined as Factor B, all documented crop-management operations were applied uniformly across the experimental subplots.

Winter wheat was harvested at maturity on 4 July 2022 using a Wintersteiger experimental plot combine. Each subplot was harvested separately from a central net area of 12.5 m² (10 m × 1.25 m). Grain yield was determined separately for each subplot in each replicate block, converted to kg ha⁻¹, and adjusted to a standard grain moisture content of 14%.

2.5. Combined N–P Mineral Fertilization Regime

Combined N–P mineral fertilization represented Factor B of the split-plot experiment and comprised two regimes: with mineral fertilizer and without mineral fertilizer. In the mineral-fertilized subplots, a granular compound fertilizer containing 18% N and 46% P₂O₅ (18–46–0; Azomureș, Târgu Mureș, Romania) was applied during autumn seedbed preparation at a rate of 150 kg ha⁻¹. This application supplied 27 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹. The compound fertilizer was incorporated into the upper 10–12 cm of soil during seedbed preparation.

Calcium ammonium nitrate (CAN; 26% N; Azomureș, Târgu Mureș, Romania) was applied as a spring top-dressing at a rate of 500 kg ha⁻¹ on 22 March 2022. The entire quantity was applied in a single dose and supplied 130 kg N ha⁻¹. Consequently, the complete mineral fertilization regime supplied a total of 157 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹.

The subplots without mineral fertilizer received no mineral fertilizer during the 2021/2022 experimental season. However, these subplots should not be interpreted as receiving no nutrient inputs because nutrients may have been introduced through the incorporated green manure crops or spontaneous vegetation.

The fertilizer rates were selected according to the locally established fertilization practice for winter cereals and the results of previous long-term fertilizer experiments conducted by the Fertilizers and Soil Amendments Laboratory at SCDA Livada. Potassium fertilizer was not included because potassium application had not produced measurable yield responses in the station's previous long-term fertilizer experiments under comparable soil conditions. The present experiment did not independently test the crop response to potassium, and no general conclusion regarding potassium requirements can therefore be drawn from this study.

Because nitrogen and phosphorus were applied together in a single combined mineral-fertilization regime, the experimental design permits evaluation only of the overall response to combined N–P fertilization. It does not permit separation of the individual effects of nitrogen and phosphorus or assessment of an N × P interaction. Furthermore, because only one combined fertilizer rate and a treatment without mineral fertilizer were included, the experiment cannot determine the optimum fertilizer rate or demonstrate that green manure reduced the mineral nitrogen requirement of winter wheat. These questions require future experiments including separate N, P, and N + P treatments and several nitrogen application rates.

2.6. Weed Management

Weed infestation was assessed once, immediately before post-emergence herbicide application, using the procedure described in Section 2.8. Following this assessment, all winter wheat subplots were treated with Sekator Progress OD at a rate of 0.15 L ha⁻¹. The herbicide contained iodosulfuron-methyl-sodium (25 g L⁻¹), amidosulfuron (100 g L⁻¹), and mefenpyr-diethyl (250 g L⁻¹) as a safener. The treatment was applied on 21 April 2022, when winter wheat was at the end of tillering to the beginning of stem elongation (BBCH 29–30).

The same herbicide treatment, application rate, and application date were used in all experimental subplots. No post-treatment assessment of weed density, weed biomass, weed

regrowth, or herbicide efficacy was conducted. Therefore, the weed data describe only the initial infestation before herbicide application and cannot be used to evaluate season-long weed suppression, herbicide efficacy, reductions in herbicide use, or herbicide resistance.

2.7. Meteorological Conditions During the 2021/2022 Experimental Period

Monthly mean air temperature and total precipitation were recorded by the SCDA Livada weather station from July 2021, when the green manure crops were established, until July 2022, when winter wheat was harvested. The monthly values recorded during the experimental period were compared with the available long-term monthly means for the same location (Table 1). The exact reference period used to calculate the long-term means was not specified in the meteorological records available for this study.

Table 1. Monthly air temperature and precipitation during the experimental period compared with the available long-term means recorded at SCDA Livada.

Month	Experimental Temperature (°C)	Long-Term Temperature (°C)	Temperature Deviation (°C)	Experimental Precipitation (mm)	Long-Term Precipitation (mm)	Precipitation Deviation (mm)
July 2021	23.4	20.5	+2.9	82.4	80.8	+1.6
August 2021	19.9	19.9	0.0	99.1	74.3	+24.8
September 2021	17.3	15.5	+1.8	100.1	66.0	+34.1
October 2021	12.1	9.8	+2.3	117.1	54.4	+62.7
November 2021	4.4	4.8	−0.4	16.4	57.4	−41.0
December 2021	4.5	0.1	+4.4	85.9	62.8	+23.1
January 2022	−1.8	−2.0	+0.2	76.6	49.3	+27.3
February 2022	2.8	0.1	+2.7	46.1	44.2	+1.9
March 2022	4.4	4.7	−0.3	1.3	45.7	−44.4
April 2022	8.9	10.5	−1.6	86.9	50.9	+36.0
May 2022	16.3	15.8	+0.5	17.9	75.5	−57.6
June 2022	22.1	19.0	+3.1	10.3	90.8	−80.5
July 2022	22.9	20.5	+2.4	36.8	80.8	−44.0

Note: Temperature deviations were calculated as the monthly experimental value minus the corresponding long-term monthly mean. Precipitation deviations were calculated using the same procedure. Positive values indicate conditions above the long-term mean, whereas negative values indicate conditions below the long-term mean. The exact reference period used to calculate the long-term means was not specified in the meteorological records available for this study.

The first part of the experimental period was generally warmer and wetter than the corresponding long-term means. July, September, and October 2021 were 2.9, 1.8, and 2.3 °C warmer than the long-term means, respectively, while precipitation in September and October exceeded the corresponding means by 34.1 and 62.7 mm. December 2021 was 4.4 °C warmer than the long-term mean. In contrast, marked precipitation deficits occurred during several important stages of winter wheat development. March 2022 received only 1.3 mm of precipitation, representing 44.4 mm less than the long-term mean. Precipitation deficits were also recorded in May, June, and July 2022, amounting to 57.6, 80.5, and 44.0 mm, respectively. June and July 2022 were also 3.1 and 2.4 °C warmer than the corresponding long-term means. These data characterize the specific meteorological conditions under which the treatment responses were observed, without establishing direct causal relationships between individual weather variables and crop performance.

2.8. Pre-Herbicide Weed Density Assessment

Weed density was assessed once in each experimental subplot immediately before the uniform post-emergence herbicide application described in Section 2.6. A 1 m² quadrat was placed within each subplot in an area considered visually representative of an intermediate level of weed infestation. Quadrat placement was based on visual assessment and was not randomized. Weeds were identified to genus or species level by the Weed Control Laboratory of SCDA Livada, and the number of individuals belonging to each taxon was

recorded. Total weed density was calculated as the sum of all weed individuals counted within the quadrat and was expressed as plants m^{-2} .

The experimental subplot, rather than the 1 m^2 quadrat, was regarded as the experimental unit. The quadrat provided one internal observation for each subplot, resulting in three subplot-level observations for each green manure treatment $\times$ mineral fertilization combination. Because only one non-randomly positioned quadrat was assessed per subplot, the weed-density results were analyzed and reported descriptively. No inferential statistical comparisons among treatments were performed for weed density. Weed cover, weed biomass, post-herbicide weed density, weed regrowth, and season-long weed suppression were not assessed.

2.9. Grain Yield and Physical Grain-Trait Measurements

At physiological maturity, winter wheat was harvested separately from a net area of 12.5 m^2 within each experimental subplot using a Wintersteiger experimental plot combine. Grain yield was recorded independently for each subplot, converted to kg ha^{-1} , and adjusted to a standard grain moisture content of 14%. The moisture-adjusted yield was calculated using the following Equation (1):

$$Y_{14} = Y_m \times \frac{100 - M}{100 - 14} \quad (1)$$

where Y_{14} is grain yield adjusted to 14% moisture, Y_m is the measured grain yield, and M is the grain moisture content at harvest (%).

One representative grain sample was collected from each experimental subplot, resulting in 36 subplot-level grain samples corresponding to six green manure treatments, two mineral fertilization levels, and three replicate blocks. Each subplot constituted one experimental unit; therefore, the grain sample collected from that subplot was used to characterize that experimental unit. Grain moisture content and hectoliter weight were determined at the SCDA Livada laboratory using a Pfeuffer Granolyser HL analyzer. Thousand-kernel weight (TKW) was determined by counting 1000 representative kernels from each sample and weighing them using a laboratory balance. Grain moisture content was expressed as a percentage (%), hectoliter weight as kg hL^{-1} , and TKW as grams (g).

2.10. Statistical Analysis

Grain yield, grain moisture content, thousand-kernel weight (TKW), and hectoliter weight were analyzed using analysis of variance (ANOVA) for a two-factor split-plot design with three replicate blocks. Green manure treatment (factor A; six levels) was assigned to the main plots, whereas the mineral fertilization regime (factor B; two levels: without mineral fertilizer and combined N–P mineral fertilization) was applied to the subplots. Green manure treatment and mineral fertilization were treated as fixed effects, and replicate block was treated as a random effect. The statistical model included the effects of replicate block, green manure treatment, the replicate block $\times$ green manure interaction, mineral fertilization, and the green manure $\times$ mineral fertilization interaction.

The effect of green manure treatment was tested against the replicate block $\times$ green manure interaction, which represented the main-plot error term. The effects of mineral fertilization and the green manure $\times$ mineral fertilization interaction were tested against the subplot residual error. Consequently, the green manure main effect was tested using 5 and 10 degrees of freedom, whereas mineral fertilization and the green manure $\times$ mineral fertilization interaction were tested using 1 and 12 and 5 and 12 degrees of freedom, respectively. For each response variable, the F statistic, numerator and denominator degrees of freedom, and p -value were reported.

When the green manure $\times$ mineral fertilization interaction was not statistically significant, the main effects were interpreted using the marginal means averaged across the levels of the other factor. When the interaction was statistically significant, the interpretation was based on simple effects. Green manure treatments receiving combined N–P mineral fertilization were compared only with the corresponding mineral-fertilized non-sown control, whereas treatments without mineral fertilizer were compared only with the corresponding non-mineral-fertilized control. The effect of mineral fertilization was also evaluated within each green manure treatment. Comparisons between treatments that differed simultaneously in both green manure type and mineral fertilization regime were not used for biological interpretation.

Pairwise comparisons were performed using Fisher's protected least significant difference (LSD) procedure only after a statistically significant omnibus F-test for the corresponding effect. The number of comparisons was restricted to biologically relevant simple comparisons defined by the experimental design: each green manure treatment versus the non-sown control at the same mineral fertilization level and the comparison between the two mineral fertilization regimes within each green manure treatment. Treatment means are presented together with their standard errors (SE), calculated from the error term appropriate to the corresponding experimental factor. Statistical significance was assessed at $p < 0.05$. Exact p -values are reported for the overall model effects, except when $p < 0.001$.

The green manure $\times$ mineral fertilization interaction was statistically significant for grain yield and TKW; therefore, simple effects were examined for these variables. The interaction was not statistically significant for grain moisture content or hectoliter weight; consequently, the main effects were interpreted for these variables. Weed-density observations were summarized descriptively and were not subjected to inferential statistical analysis because only one non-randomly positioned quadrat was assessed in each subplot before herbicide application.

3. Results

3.1. Pre-Herbicide Weed Density

The weed flora observed immediately before post-emergence herbicide application included *Cirsium arvense* (L.) Scop., *Tripleurospermum odoratum* (L.) Sch.Bip., *Viola arvensis* Murray, and *Capsella bursa-pastoris* (L.) Medik. Because species-specific abundance, weed cover, and weed biomass were not quantified, the results are presented only as total pre-herbicide weed density. The mean pre-herbicide weed densities recorded for the green manure treatments and the non-sown control with spontaneous vegetation are presented descriptively in Figure 3. Each value represents the mean of three subplot-level observations, one from each replicate block. The non-sown control with spontaneous vegetation had mean densities of 80 plants m^{-2} under combined N–P mineral fertilization and 75 plants m^{-2} without mineral fertilizer. Among the green manure treatments, triticale had the lowest numerical values, with 33 and 22 plants m^{-2} under combined N–P mineral fertilization and without mineral fertilizer, respectively. Lupine had the highest numerical values among the sown green manure treatments, with 55 and 54 plants m^{-2} , respectively. No inferential statistical comparisons were performed for weed density; therefore, these numerical differences should not be interpreted as statistically demonstrated treatment effects.

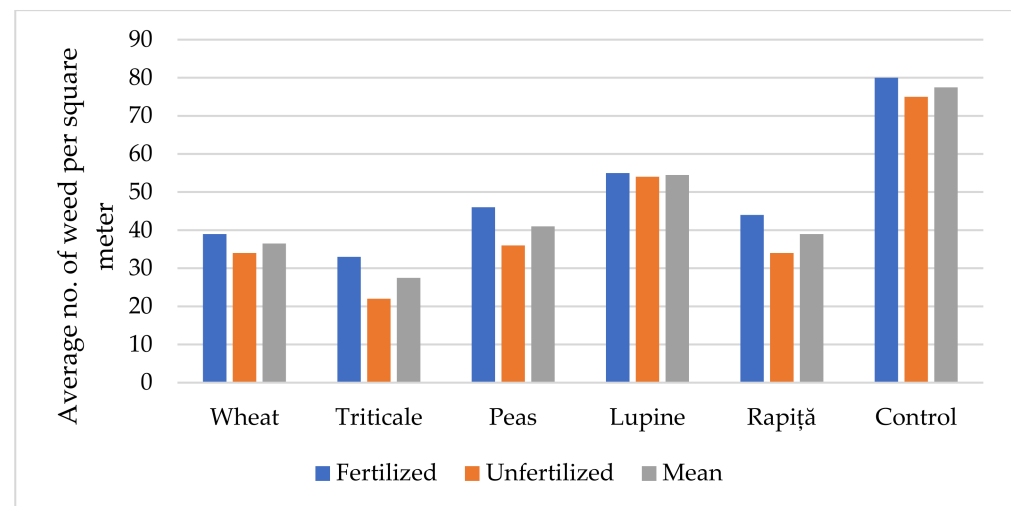


Figure 3. Mean pre-herbicide weed density in winter wheat following the green manure treatments and the non-sown control with spontaneous vegetation, under combined N–P mineral fertilization and without mineral fertilizer. Values represent means of three subplot-level observations ($n = 3$) and are expressed as plants m^{-2} . The data are presented descriptively; no inferential statistical comparisons were performed.

3.2. Wheat Grain Yield

The split-plot ANOVA showed significant effects of green manure treatment ($F_{5,10} = 111.02$, $p < 0.001$), combined N–P mineral fertilization ($F_{1,12} = 494.07$, $p < 0.001$), and their interaction ($F_{5,12} = 5.44$, $p = 0.008$) on winter wheat grain yield (Table 2). Because the green manure $\times$ mineral fertilization interaction was significant, the interpretation was based primarily on simple effects, namely comparisons among green manure treatments within the same mineral fertilization regime and comparisons between the two mineral fertilization regimes within each green manure treatment.

Table 2. Split-plot analysis of variance for winter wheat grain yield and physical grain traits.

Response Variable	Source of Variation	df	F	p-Value
Grain yield ($kg\ ha^{-1}$)	Green manure treatment (A)	5, 10	111.02	<0.001
	Mineral fertilization (B)	1, 12	494.07	<0.001
	A $\times$ B	5, 12	5.44	0.008
Grain moisture content (%)	Green manure treatment (A)	5, 10	10.41	0.001
	Mineral fertilization (B)	1, 12	43.33	<0.001
	A $\times$ B	5, 12	0.36	0.869
Thousand-kernel weight (g)	Green manure treatment (A)	5, 10	4.96	0.015
	Mineral fertilization (B)	1, 12	6.55	0.025
	A $\times$ B	5, 12	3.46	0.036
Hectoliter weight ($kg\ hL^{-1}$)	Green manure treatment (A)	5, 10	21.72	<0.001
	Mineral fertilization (B)	1, 12	1.60	0.231
	A $\times$ B	5, 12	0.43	0.817

Note: For the green manure main effect, the numerator and denominator degrees of freedom were 5 and 10, respectively, because the replicate block $\times$ green manure interaction was used as the main-plot error term. Mineral fertilization and the A $\times$ B interaction were tested against the subplot residual error. Statistically significant p -values ($p < 0.05$) are shown in bold.

Under combined N–P mineral fertilization, the non-sown control with spontaneous vegetation produced $6730 \pm 244\ kg\ ha^{-1}$ (mean $\pm$ SE). Pea produced the highest numerical yield, $8018 \pm 116\ kg\ ha^{-1}$, exceeding the corresponding control by $1287\ kg\ ha^{-1}$ ($p < 0.001$). Lupine and oilseed rape produced 7560 ± 261 and $7750 \pm 152\ kg\ ha^{-1}$, respec-

tively, corresponding to increases of 830 and 1020 kg ha⁻¹ relative to the mineral-fertilized control ($p < 0.01$). In contrast, wheat and triticale green manures produced 4954 ± 274 and 5612 ± 264 kg ha⁻¹, respectively, representing reductions of 1776 and 1118 kg ha⁻¹ relative to the corresponding control ($p < 0.001$).

Without mineral fertilizer, the non-sown control with spontaneous vegetation produced 3419 ± 108 kg ha⁻¹. Pea and lupine produced 5427 ± 187 and 5009 ± 237 kg ha⁻¹, respectively, exceeding the corresponding control by 2008 and 1590 kg ha⁻¹ ($p < 0.001$). Wheat green manure produced 2242 ± 151 kg ha⁻¹, which was 1177 kg ha⁻¹ lower than the corresponding control ($p < 0.001$). The yields recorded after triticale and oilseed rape did not differ significantly from the non-mineral-fertilized control ($p \geq 0.05$).

Combined N–P mineral fertilization increased grain yield within each green manure treatment and within the non-sown control ($p < 0.001$). The largest numerical fertilization response was observed after oilseed rape, for which grain yield increased by 4541 kg ha⁻¹. The corresponding numerical increases were 2712 kg ha⁻¹ after wheat, 2597 kg ha⁻¹ after triticale, 2591 kg ha⁻¹ after pea, 2551 kg ha⁻¹ after lupine, and 3311 kg ha⁻¹ in the non-sown control with spontaneous vegetation. These results describe the response to the combined N–P fertilization regime and do not allow the separate effects of nitrogen and phosphorus to be distinguished.

The simple effects of green manure treatment within each mineral fertilization regime are presented in Table 3.

Table 3. Simple effects of green manure treatment and combined N–P mineral fertilization on winter wheat grain yield.

Green Manure Treatment	Combined N–P Mineral Fertilization (kg ha ⁻¹)	Difference from Corresponding Control (kg ha ⁻¹)	Without Mineral Fertilizer (kg ha ⁻¹)	Difference from Corresponding Control (kg ha ⁻¹)	Fertilization Response (kg ha ⁻¹)
Wheat	4954 ± 274	−1776 ***	2242 ± 151	−1177 ***	+2712 ***
Triticale	5612 ± 264	−1118 ***	3016 ± 196	−403 ns	+2597 ***
Pea	8018 ± 116	+1287 ***	5427 ± 187	+2008 ***	+2591 ***
Narrow-leafed lupine	7560 ± 261	+830 **	5009 ± 237	+1590 ***	+2551 ***
Oilseed rape	7750 ± 152	+1020 **	3209 ± 95	−210 ns	+4541 ***
Non-sown control with spontaneous vegetation	6730 ± 244	—	3419 ± 108	—	+3311 ***

Note: Values are means $\pm$ SE based on three replicate blocks. Within each mineral fertilization regime, green manure treatments were compared only with the corresponding non-sown control with spontaneous vegetation. The fertilization response represents the difference between combined N–P mineral fertilization and the treatment without mineral fertilizer within the same green manure treatment. Pairwise comparisons were performed using Fisher's protected LSD following a significant omnibus F-test. ** and *** indicate significance at $p < 0.01$ and $p < 0.001$, respectively; ns indicates $p \geq 0.05$. Differences were calculated using the unrounded treatment means, whereas the displayed means and differences were rounded to the nearest kg ha⁻¹.

Grain moisture content at harvest was significantly affected by green manure treatment ($F_{5,10} = 10.41$, $p = 0.001$) and combined N–P mineral fertilization ($F_{1,12} = 43.33$, $p < 0.001$). However, the green manure $\times$ mineral fertilization interaction was not significant ($F_{5,12} = 0.36$, $p = 0.869$) (Table 2). Consequently, the effects of green manure treatment and mineral fertilization were interpreted using marginal means rather than comparisons among individual treatment combinations.

Averaged across the two mineral fertilization regimes, grain moisture content ranged numerically from 10.65% after pea to 11.53% after wheat green manure (Table 4). The corresponding marginal means were 10.88% after narrow-leafed lupine, 11.05% after oilseed rape, 11.20% after triticale, and 11.14% for the non-sown control with spontaneous vegetation. Because no adjusted pairwise comparisons among these marginal means were performed, the observed differences are reported as numerical patterns and should not be interpreted as statistically confirmed differences between individual green manure treatments.

Table 4. Grain moisture content at harvest according to green manure treatment and mineral fertilization regime.

Green Manure Treatment	Combined N–P Mineral Fertilization (%)	Without Mineral Fertilizer (%)	Marginal Mean (%)
Wheat	11.23	11.83	11.53
Triticale	10.83	11.57	11.20
Pea	10.37	10.93	10.65
Narrow-leaved lupine	10.53	11.23	10.88
Oilseed rape	10.57	11.53	11.05
Non-sown control with spontaneous vegetation	10.67	11.60	11.14
Mean across green manure treatments	10.70	11.45	—

Note: Values are means based on three replicate blocks. The marginal mean for each green manure treatment was calculated across the two mineral fertilization regimes. The green manure $\times$ mineral fertilization interaction was not significant ($F_{5,12} = 0.36$, $p = 0.869$); therefore, individual treatment combinations were not subjected to simple-effect interpretation. The main effect of mineral fertilization was significant ($F_{1,12} = 43.33$, $p < 0.001$). Differences between moisture values are expressed in percentage points, not as relative percentages.

Averaged across all green manure treatments, grain moisture content was 10.70% under combined N–P mineral fertilization and 11.45% without mineral fertilizer. Thus, combined N–P mineral fertilization was associated with an average reduction of 0.75 percentage points in grain moisture content at harvest ($p < 0.001$). The nonsignificant interaction indicates that the direction of the fertilization response did not differ statistically among the green manure treatments.

3.3. Thousand-Kernel Weight

Thousand-kernel weight (TKW) was significantly affected by green manure treatment ($F_{5,10} = 4.96$, $p = 0.015$), combined N–P mineral fertilization ($F_{1,12} = 6.55$, $p = 0.025$), and the green manure $\times$ mineral fertilization interaction ($F_{5,12} = 3.46$, $p = 0.036$) (Table 2). Because the interaction was significant, the interpretation was based on comparisons among green manure treatments within the same mineral fertilization regime and on the effect of mineral fertilization within each green manure treatment.

Under combined N–P mineral fertilization, wheat and triticale produced TKW values of 41.93 and 41.87 g, respectively, exceeding the corresponding mineral-fertilized control value of 40.80 g by 1.13 and 1.07 g ($p < 0.05$). Pea, narrow-leaved lupine, and oilseed rape did not differ significantly from the mineral-fertilized control ($p \geq 0.05$).

Without mineral fertilizer, pea, narrow-leaved lupine, and oilseed rape produced TKW values of 42.87, 43.00, and 42.73 g, respectively. These values exceeded the corresponding non-mineral-fertilized control value of 41.13 g by 1.73, 1.87, and 1.60 g, respectively ($p < 0.01$). Wheat and triticale did not differ significantly from the corresponding control ($p \geq 0.05$).

The response to combined N–P mineral fertilization differed among green manure treatments. Mineral fertilization was associated with lower TKW after pea, narrow-leaved lupine, and oilseed rape. The reduction was 2.27 g after pea ($p < 0.001$) and 1.33 g after both narrow-leaved lupine and oilseed rape ($p < 0.05$). The fertilized and non-mineral-fertilized variants did not differ significantly after wheat or triticale or in the non-sown control with spontaneous vegetation ($p \geq 0.05$).

The simple effects of green manure treatment within each mineral fertilization regime on thousand-kernel weight are presented in Table 5.

Table 5. Simple effects of green manure treatment and combined N–P mineral fertilization on winter wheat thousand-kernel weight.

Green Manure Treatment	Combined N–P Mineral Fertilization (g)	Difference from Corresponding Control (g)	Without Mineral Fertilizer (g)	Difference from Corresponding Control (g)	Fertilization Response (g)
Wheat	41.93	+1.13 *	41.13	0.00 ns	+0.80 ns
Triticale	41.87	+1.07 *	41.40	+0.27 ns	+0.47 ns
Pea	40.60	−0.20 ns	42.87	+1.73 **	−2.27 ***
Narrow-leafed lupine	41.67	+0.87 ns	43.00	+1.87 **	−1.33 *
Oilseed rape	41.40	+0.60 ns	42.73	+1.60 **	−1.33 *
Non-sown control with spontaneous vegetation	40.80	—	41.13	—	−0.33 ns

Note: Values are means based on three replicate blocks; the model-based standard error of a treatment-combination mean was approximately 0.31 g. Within each mineral fertilization regime, green manure treatments were compared only with the corresponding non-sown control with spontaneous vegetation. The fertilization response represents the difference between combined N–P mineral fertilization and the treatment without mineral fertilizer within the same green manure treatment. Pairwise comparisons were performed using Fisher’s protected LSD following a significant omnibus F-test. *, **, and *** indicate significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns indicates $p \geq 0.05$. Differences were calculated using the unrounded treatment means, whereas the displayed means and differences were rounded to two decimal places.

3.4. Hectoliter Weight

Hectoliter weight was significantly affected by green manure treatment ($F_{5,10} = 21.72$, $p < 0.001$). However, neither the main effect of combined N–P mineral fertilization ($F_{1,12} = 1.60$, $p = 0.231$) nor the green manure $\times$ mineral fertilization interaction ($F_{5,12} = 0.43$, $p = 0.817$) was statistically significant (Table 2). Consequently, the effect of green manure treatment was interpreted using marginal means averaged across the two mineral fertilization regimes.

Averaged across mineral fertilization regimes, the non-sown control with spontaneous vegetation produced a hectoliter weight of 83.10 kg hL^{−1} (Table 6). Wheat and triticale green manures produced values of 81.48 and 81.39 kg hL^{−1}, corresponding to reductions of 1.62 and 1.72 kg hL^{−1}, respectively, relative to the control ($p < 0.01$).

Table 6. Main effect of green manure treatment on winter wheat hectoliter weight, averaged across mineral fertilization regimes.

Green Manure Treatment	Hectoliter Weight (kg hL ^{−1})	Difference from Control (kg hL ^{−1})
Wheat	81.48 ± 0.30	−1.62 **
Triticale	81.39 ± 0.30	−1.72 **
Pea	84.42 ± 0.30	+1.32 *
Narrow-leafed lupine	84.22 ± 0.30	+1.12 *
Oilseed rape	84.27 ± 0.30	+1.17 *
Non-sown control with spontaneous vegetation	83.10 ± 0.30	—

Note: Values are marginal means $\pm$ model-based SE, averaged across the two mineral fertilization regimes and based on three replicate blocks. Green manure treatments were compared with the non-sown control with spontaneous vegetation using Fisher’s protected LSD following the significant omnibus F-test for the green manure main effect. * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively. Differences were calculated using unrounded means. The main effect of mineral fertilization and the green manure $\times$ mineral fertilization interaction were not statistically significant.

Pea, narrow-leafed lupine, and oilseed rape produced hectoliter weights of 84.42, 84.22, and 84.27 kg hL^{−1}, respectively. These values exceeded the control by 1.32, 1.12, and 1.17 kg hL^{−1}, respectively ($p < 0.05$).

Averaged across all green manure treatments, hectoliter weight was 83.38 kg hL⁻¹ under combined N–P mineral fertilization and 82.91 kg hL⁻¹ without mineral fertilizer. This numerical difference of 0.47 kg hL⁻¹ was not statistically significant ($p = 0.231$). The nonsignificant interaction indicates that the effect of green manure treatment on hectoliter weight did not differ statistically between the two mineral fertilization regimes.

4. Discussion

The results obtained at SCDA Livada during the 2021/2022 growing season indicate that the response of subsequent winter wheat depended on both the green manure species and the mineral fertilization regime. The significant green manure $\times$ mineral fertilization interaction for grain yield ($F_{5,12} = 5.44$, $p = 0.008$) shows that the magnitude of the response to green manure differed between the two mineral fertilization regimes. Because the experiment was conducted at a single location during one growing season, the discussion is restricted to the conditions under which the study was performed.

Under combined N–P mineral fertilization, pea, narrow-leaved lupine, and oilseed rape produced significantly higher winter wheat yields than the corresponding mineral-fertilized non-sown control with spontaneous vegetation. Without mineral fertilizer, only pea and narrow-leaved lupine produced significantly higher yields than the corresponding control. Oilseed rape did not differ significantly from the control without mineral fertilizer. These findings indicate that the response associated with oilseed rape depended more strongly on the mineral fertilization regime, whereas the positive yield responses associated with pea and narrow-leaved lupine were observed under both regimes.

The higher yields observed after pea and narrow-leaved lupine are consistent with the yield responses following legume cover crops reported in previous studies and syntheses [1–3,5–7,12–15]. Leguminous species can obtain nitrogen through biological nitrogen fixation and may produce residues that release nitrogen more rapidly than cereal residues [3,4,6]. However, biological nitrogen fixation, nodulation, residue nitrogen concentration, dry biomass, C:N ratio, soil mineral nitrogen, and nitrogen uptake by the subsequent wheat crop were not measured in the present experiment. Therefore, biological nitrogen fixation and residue decomposition should be considered possible explanations supported by the literature, rather than mechanisms demonstrated by the present results.

Leguminous green manures may also influence phosphorus acquisition through root-induced chemical changes, rhizosphere processes, and interactions with soil microorganisms [13,14]. These processes could be relevant under acidic soil conditions, where phosphorus availability may be restricted. Nevertheless, neither phosphorus availability after green manure incorporation nor phosphorus uptake by winter wheat was measured. Furthermore, the mineral-fertilized treatment received nitrogen and phosphorus together. The experimental design therefore does not permit the separate contributions of nitrogen and phosphorus, or their individual interactions with green manure species, to be identified.

Wheat and triticale green manures were associated with lower subsequent wheat yields than the corresponding controls under the conditions of this experiment. Cereal residues frequently have a higher C:N ratio than legume residues and may temporarily immobilize mineral nitrogen during decomposition [5,15]. This mechanism could provide one possible explanation for the observed yield response, particularly because the green manure biomass was incorporated shortly before establishment of the subsequent wheat crop. However, residue dry matter, carbon and nitrogen concentrations, C:N ratio, decomposition rate, and soil mineral nitrogen were not determined. Consequently, nitrogen immobilization cannot be identified as the demonstrated cause of the lower yields. The findings show only that using wheat or triticale as green manure was associated with lower

subsequent wheat yield during the evaluated season; they do not establish that cereal green manures are generally unsuitable.

Combined N–P mineral fertilization increased winter wheat grain yield within every green manure treatment. The absolute response differed among treatments, ranging from approximately 2551 kg ha^{−1} after narrow-leaved lupine to 4541 kg ha^{−1} after oilseed rape. This variation is consistent with the statistically significant interaction between green manure treatment and mineral fertilization. Nevertheless, because the fertilized treatment received 157 kg N ha^{−1} and 69 kg P₂O₅ ha^{−1} together, the yield response cannot be attributed separately to nitrogen or phosphorus.

The largest numerical yield was recorded when pea green manure was combined with the N–P mineral fertilization regime. However, this result does not demonstrate that this combination is agronomically or economically optimal, because the experiment included only one mineral-fertilizer rate and did not include separate N, P, and N+P treatments or an economic analysis. Similarly, the results cannot establish that green manure reduced the mineral-nitrogen requirement or improved fertilizer-use efficiency. Testing these hypotheses would require experiments with several nitrogen and phosphorus rates, direct measurements of nutrient inputs and crop uptake, and calculations of nutrient-use efficiency.

Overall, the results are compatible with the broader concept of combining organic residue inputs with mineral fertilization [16,17]. However, soil organic matter changes, soil biological activity, nutrient losses, nutrient-use efficiency, and environmental effects were not measured. The present study therefore demonstrates treatment-related differences in winter wheat yield but does not directly demonstrate improvements in soil quality, nutrient cycling, resource-use efficiency, or environmental sustainability.

4.1. Interpretation of Pre-Herbicide Weed Density

The descriptive assessment conducted immediately before post-emergence herbicide application showed numerically higher total weed density in the non-sown control with spontaneous vegetation than in the treatments previously occupied by sown green manure crops. This numerical pattern was observed under both mineral fertilization regimes. However, weed-density data were not subjected to inferential statistical analysis because only one non-randomly positioned 1 m² quadrat was assessed in each subplot. Consequently, the observed differences cannot be interpreted as statistically demonstrated effects of the green manure treatments.

Among the sown treatments, triticale had the lowest numerical pre-herbicide weed density, whereas narrow-leaved lupine had the highest. This variation indicates that the sown green manure species should not be considered a homogeneous group. Nevertheless, species-specific weed abundance, weed cover, weed biomass, green manure canopy development, and the degree or duration of soil coverage were not quantified. Therefore, the experiment cannot determine which characteristics of the individual green manure crops were associated with the observed variation in weed density.

Previous research has shown that cover crops can influence weed establishment through competition for light, water, nutrients, and physical space and that the magnitude of weed suppression can be related to canopy development and biomass production [18,19]. Cover crop residues may also affect subsequent weed emergence, and their effects can vary according to crop species, residue quantity, residue persistence, and environmental conditions [18–20]. These mechanisms provide possible explanations for the numerical pattern observed in the present study. However, competition during green manure growth could not be separated from possible residue-mediated effects after incorporation, and allelopathic activity was not measured.

The weed assessment represented only the initial weed density before the uniform herbicide treatment. No post-herbicide assessment was performed, and weed cover, weed biomass, weed regrowth, weed seed production, and final weed density were not determined. Accordingly, the present results do not provide evidence regarding herbicide efficacy, season-long weed suppression, reduced herbicide requirements, or herbicide-resistance management. Repeated assessments using several randomly positioned quadrats per subplot, together with species-specific density, cover, and biomass measurements before and after herbicide application, would be required to evaluate these outcomes.

4.2. Effects on Physical Grain Traits

The physical grain traits showed different responses to green manure treatment and combined N–P mineral fertilization. Grain moisture content was significantly affected by green manure treatment and mineral fertilization, whereas their interaction was not significant. Averaged across the green manure treatments, combined N–P mineral fertilization was associated with a grain moisture content of 10.70%, compared with 11.45% without mineral fertilizer. The difference of 0.75 percentage points indicates that the fertilized wheat was harvested at a slightly lower mean grain moisture content.

Differences in grain moisture at harvest may be associated with variation in crop development, senescence, or the timing of grain maturation. However, phenological development, canopy senescence, and the grain-drying rate were not monitored. Consequently, the physiological mechanism underlying the observed moisture response cannot be determined from the present experiment. The practical importance of the differences was limited because all treatment means were below the standard moisture content of 14% used to adjust grain yield.

Thousand-kernel weight showed a significant green manure $\times$ mineral fertilization interaction, indicating that its response depended on the combination of the two experimental factors. Under combined N–P mineral fertilization, wheat and triticale green manures produced significantly higher TKW values than the corresponding mineral-fertilized control, whereas pea, narrow-leaved lupine, and oilseed rape did not differ significantly from that control. Without mineral fertilizer, pea, narrow-leaved lupine, and oilseed rape produced significantly higher TKW values than the corresponding non-mineral-fertilized control, whereas wheat and triticale did not differ significantly from the control.

The higher TKW observed for some treatments could reflect differences in grain filling or compensation between grain number and individual kernel weight. Previous research has shown that kernel weight can be influenced by assimilate availability and by the relationship between grain number and grain filling [21]. However, spike density, grain number per spike, total grain number per unit area, and grain-filling duration were not measured in the present study. Therefore, the physiological basis of the TKW response remains unresolved.

Hectoliter weight was significantly affected by green manure treatment, whereas neither the mineral fertilization main effect nor the green manure $\times$ mineral fertilization interaction was significant. Averaged across the two mineral fertilization regimes, wheat and triticale green manures were associated with significantly lower hectoliter weights than the non-sown control with spontaneous vegetation. In contrast, pea, narrow-leaved lupine, and oilseed rape were associated with significantly higher hectoliter weights than the control.

Although nitrogen nutrition can influence wheat grain development and physical or technological grain properties [22], the present experiment did not show a statistically significant effect of the combined N–P mineral fertilization regime on hectoliter weight. Moreover, grain protein concentration, gluten content, test-baking properties,

and other technological-quality indicators were not measured. The results should therefore be restricted to the physical grain traits evaluated and should not be interpreted as demonstrating a general improvement in wheat grain quality.

Overall, the treatment effects were more pronounced for grain yield than for the physical grain traits. The responses of grain moisture, TKW, and hectoliter weight were not uniform: grain moisture showed significant main effects without a significant interaction, TKW showed a significant interaction, and hectoliter weight was influenced only by the green manure main effect. Consequently, a general claim that leguminous green manures improved all aspects of grain quality would not be supported by the results.

4.3. Agronomic Implications, Study Limitations and Future Research

Under the soil and climatic conditions of the 2021/2022 experiment at SCDA Livada, pea and narrow-leaved lupine showed the most favorable yield responses among the evaluated green manure crops. Both species produced significantly higher subsequent winter wheat yields than the corresponding non-sown control, with spontaneous vegetation under combined N–P mineral fertilization and without mineral fertilizer. Oilseed rape produced a significantly higher yield than that of the corresponding control only under combined N–P mineral fertilization. These results identify pea and narrow-leaved lupine as promising candidates for additional evaluation under the acidic, low-fertility conditions represented by the experimental site.

Wheat and triticale used as green manure were associated with lower subsequent winter wheat yields than the corresponding controls under several treatment conditions. This finding is agronomically relevant because the use of wheat as green manure before winter wheat represents an atypical crop sequence and may involve residue-management, nutrient, or phytosanitary constraints. However, residue decomposition, nitrogen immobilization, soil mineral nitrogen, pathogens, and disease incidence were not measured. The results therefore do not demonstrate that wheat and triticale are generally unsuitable as green manure crops. They show only that their use was associated with lower subsequent wheat yields under the conditions of this single-season experiment.

The experiment has several important limitations. First, it was conducted at one location during one growing season using three replicate blocks. Consequently, interannual climatic variability and the stability of the treatment responses across different locations and soil-fertility conditions could not be evaluated. The results should therefore not be generalized beyond the soil, climatic, and management conditions represented by the experiment.

Second, green manure inputs were characterized using fresh biomass. Dry matter content, residue carbon and nitrogen concentrations, C:N ratio, total nitrogen input, decomposition rate, and nutrient release were not determined. Although the roots collected during biomass sampling contributed to the recorded fresh mass, belowground biomass production was not characterized separately. Differences among green manure species therefore cannot be attributed quantitatively to the amount or biochemical quality of the incorporated residues.

Third, the non-sown control contained spontaneous vegetation that was incorporated into the soil. Although the principal weed taxa were identified, the fresh and dry biomass, botanical composition by relative abundance, carbon and nitrogen concentrations, and nutrient contribution of this vegetation were not quantified separately. Consequently, the control represented a non-sown treatment with spontaneous vegetation rather than a treatment without plant-derived organic inputs.

Fourth, the mineral fertilization factor included only two regimes: without mineral fertilizer and one combined N–P treatment supplying 157 kg N ha^{−1} and 69 kg P₂O₅ ha^{−1}.

The design did not include separate N, P, and N+P treatments, intermediate fertilizer rates, or potassium fertilization. Therefore, the individual effects of nitrogen and phosphorus could not be separated, an economically optimal fertilizer rate could not be calculated, and the experiment could not determine whether green manure reduced the mineral-nitrogen requirement of winter wheat. Furthermore, the treatments without mineral fertilizer should not be interpreted as receiving no nutrient input because nutrients may have been supplied by the incorporated plant material.

Fifth, weed density was evaluated only once, immediately before herbicide application, using one non-randomly positioned quadrat per subplot. Weed cover, weed biomass, post-herbicide density, regrowth, and seed production were not evaluated. The weed observations therefore provide only a descriptive indication of initial pre-herbicide density and cannot support conclusions regarding herbicide efficacy, season-long weed control, reduced herbicide use, or herbicide-resistance management.

Finally, soil mineral nitrogen, plant nutrient uptake, biological nitrogen fixation, soil phosphorus availability, soil biological activity, greenhouse-gas emissions, nutrient losses, changes in soil chemical and physical properties, and the economic performance of the treatments were not measured. Accordingly, the study cannot demonstrate improved nutrient cycling, fertilizer-use efficiency, soil quality, environmental performance, or overall system sustainability.

Future research should evaluate the green manure species across multiple growing seasons, locations, and contrasting soil-fertility levels, including more fertile soils. Such experiments should include separate no-fertilizer, N, P, and N+P treatments, together with several intermediate nitrogen rates, to determine crop-specific fertilizer responses and test whether green manure can reduce mineral-nitrogen requirements. Future studies should also quantify fresh and dry aboveground and belowground biomass, residue carbon and nitrogen concentrations, C:N ratio, biological nitrogen fixation, nutrient release, soil nutrient dynamics, crop nutrient uptake, and changes in soil properties. Repeated and randomized weed assessments, together with weed density, species composition, cover, and biomass measurements before and after herbicide application, would permit a more rigorous evaluation of weed dynamics. Economic analysis would also be required before practical recommendations regarding the most advantageous green manure and fertilization combinations could be made.

5. Conclusions

In this single-location experiment conducted during the 2021/2022 growing season on acidic, low-fertility soil at SCDA Livada, winter wheat grain yield was significantly affected by green manure treatment, combined N–P mineral fertilization, and their interaction. Pea and narrow-leaved lupine produced significantly higher subsequent wheat yields than the corresponding non-sown control with spontaneous vegetation under both mineral fertilization regimes. Oilseed rape produced a significantly higher yield than the corresponding control only when combined N–P mineral fertilization was applied.

Wheat green manure was associated with significantly lower subsequent wheat yield than the corresponding control under both mineral fertilization regimes. Triticale produced a significantly lower yield than the control under combined N–P mineral fertilization, whereas it did not differ significantly from the control without mineral fertilizer. These findings are specific to the conditions and crop sequence evaluated and do not demonstrate the general unsuitability of cereal green manures.

Combined N–P mineral fertilization significantly increased grain yield within every green manure treatment. The highest numerical yield, 80,188,018 kg ha^{−1}, was obtained when pea green manure was combined with mineral fertilization. However, because only

one combined N–P fertilizer rate was evaluated, the individual effects of nitrogen and phosphorus could not be separated, and neither an optimal fertilizer rate nor a reduction in mineral-nitrogen requirements could be determined.

The physical grain traits did not respond uniformly. Grain moisture content was significantly affected by green manure treatment and mineral fertilization, but their interaction was not significant. Thousand-kernel weight showed a significant green manure $\times$ mineral fertilization interaction, whereas hectoliter weight was significantly influenced only by green manure treatment. Therefore, the results do not support a general conclusion that any botanical group consistently improved all physical grain traits.

Pre-herbicide weed density was numerically higher in the non-sown control with spontaneous vegetation than following the sown green manure treatments. However, these observations were descriptive and based on a single non-randomly positioned quadrat per subplot. Consequently, no conclusions can be drawn regarding statistically significant weed suppression, herbicide efficacy, season-long weed control, or reduced herbicide requirements.

Overall, pea and narrow-leafed lupine showed the most favorable yield responses under the conditions of this experiment and warrant further evaluation as green manure crops for acidic, low-fertility soils. Multi-year and multi-location experiments, including contrasting soil-fertility conditions, several nitrogen and phosphorus rates, and direct measurements of dry biomass, residue composition, nutrient inputs, soil properties, weed dynamics, and economic performance, are required before broader agronomic or sustainability recommendations can be made.

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