

Article

Optimal Nitrogen Fertilizer Rates for Soybean Cultivation

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Abstract: The soybean (*Glycine max.* L. Merr) can satisfy a large portion of its requirement for nitrogen (N) by living in symbiosis with symbiotic bacteria. However, this source of N may be inadequate in varieties with high yield potential. To fully exploit this potential, soybeans should additionally utilize mineral forms of nitrogen present in the soil. The aim of this study was to determine the effect of varied nitrogen fertilizer application rates on the dry weight of the separated parts of soybean plants and the whole plant, including the number and weight of root nodules, the potential to reduce atmospheric nitrogen (N₂), and the content and uptake of nitrogen. Four levels of pre-sowing nitrogen fertilizer supply were tested: 0, 60, 120, and 180 kg N·ha^{−1}. Measurements of the tested parameters were taken during the flowering stage and the fully ripe stage. During the flowering stage, a reduction in the number of root nodules was observed following the application of 120 and 180 kg N·ha^{−1}. In the fully ripe stage, each increase in nitrogen application caused a systematic decrease in the number of nodules on the roots. Increasing the level of nitrogen application therefore reduced the N₂ fixation potential of soybeans, regardless of the developmental stage. The use of high doses of nitrogen in soybean cultivation did not increase seed yield or the weight of the entire plant. With high doses of nitrogen, the content and accumulation of nitrogen in soybean seeds and total mass did not increase. Therefore, the content and yield of crude protein did not increase. The main organ of nitrogen accumulation in the soybean flowering stage was the leaves (58.6–64.8% of total N uptake), however, in the fully ripe stage, it was the seeds (66.8–74.2% of total N uptake).

Keywords: nitrogen content; nitrogen uptake; root nodules; soybean; yield



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1. Introduction

Agriculture, apart from its production functions, should protect landscape biodiversity and the quality of the air, water, and soil. Its intensification in order to meet the increased demands for food production is associated with changes in production technology, the introduction of new species, and the appearance of large-scale monoculture crops. These conditions lead to a reduction in the landscape properties of these areas and also negatively affect the diversity of the flora and fauna of agricultural ecosystems [1]. Sustainable protein crop production and profitable yield is considered a key issue for European agriculture in order to meet the high demands of the feed industry and added-value markets. In Poland, high demand for feed led to a more than 20-fold increase in the share of maize crops in the agricultural landscape between 1990 and 2022 [2]. The increasing demand for protein for food and fodder has led to efforts to expand soybean cultivation in Poland. In 2008, soybeans in Poland occupied an area of only 67 ha, but this increased to more than 18,000 ha by 2022 [2]. On a global scale, soybeans are a popular crop plant whose cultivation area nearly doubled between 2000 and 2022 (from more than 74 million ha to

more than 133 million ha) [2]. At the same time, the cultivation area in Europe increased about sixfold (from more than 1 million ha to more than 6 million ha) [2].

The soybean plant is one of the most important oilseed plants around the world which additionally yields a large amount of protein of high nutritional value for human food and animal feed, whereas, as an energy crop, it is sold for biodiesel production [3–8]. Economic success in its cultivation requires not only the exploitation of the natural productivity of the site, but also large inputs of industrial means of production, e.g., plant protection products, fertilizers, and irrigation [9,10]. Non-market co-benefits, such as the nitrogen (N_2) fixation and accumulation in the soil for the cultivation of subsequent crops, should also be included in the cost-benefit analysis of soybean production [11]. Among fertilizers, nitrogen (N) fertilizers are well known to contribute the most to increasing the weight of plants, including seed yield [11]. However, the use of excessively high amounts of these fertilizers can contribute to environmental pollution through the escape of gaseous forms into the atmosphere (NH_3 , NO, N_2O , greenhouse effect), their accumulation in surface water (eutrophication) and groundwater (nitrate contamination of drinking water), or N lost from soil surface runoff, ultimately having a negative impact on human health [12–15]. Of the total amount of nitrogen introduced to the soil, about 50% is utilized by plants, 20% is immobilized, and 25% is lost (20% escapes in a gaseous form to the atmosphere as NH_3 or NO_x and 5% is leached into the soil profile) [16]. Legume plants, which include soybeans, generally do not require copious nitrogen fertilizer application because they can live in symbiosis with rhizobia, leading to the reduction of atmospheric N_2 forms available for the host plant. They require only small amounts of available nitrogen at the start of the growing period. In the later period of growth and development, the atmosphere becomes their source of this macronutrient [17–19]. Owing to symbiosis, about 30–60% of the nitrogen requirement of the soybean plant can be met, and, in this situation, in the case of yield not exceeding $3\text{ Mg}\cdot\text{ha}^{-1}$, supplementary mineral fertilization in the amount of about $30\text{ kg N}\cdot\text{ha}^{-1}$ before sowing is sufficient [20]. The uptake of atmospheric N_2 is an energy-intensive process for the host plant, as reduction of one mole of N_2 requires at least 8 moles of electrons and 16 moles of ATP, which must be supplied by the plant [21]. For this reason, a plant growing in conditions with large amounts of mineral forms of nitrogen more readily uses them as a less costly source for the plant, at the same time limiting symbiosis with microorganisms.

Data collected from a period of more than half a century indicate that as the amount of nitrogen biologically reduced by the soybean plant has increased, the seed yield per unit of N_2 fixation has decreased from 0.033 to $0.017\text{ Mg yield kg}^{-1}\text{ N}$, i.e., moving from low (28%) to high (80%) levels of nitrogen derived from the atmosphere [22]. This suggests the need for the application of additional N to exploit the yield potential of the plant [22]. LaMenza et al. [23] showed that soybean yield is limited by the supply in the environment, and for a soybean plant to produce a seed yield from 6 to $8\text{ Mg}\cdot\text{ha}^{-1}$, it should take up 480 to 640 kg N ha^{-1} ($80\text{ kg N}\cdot\text{Mg}^{-1}$ seeds). They also indicated that soybeans grown in conditions enabling yield at a level above $2.5\text{ Mg}\cdot\text{ha}^{-1}$ may react positively to nitrogen application. Unfortunately, high concentrations of mineral forms of nitrogen in the soil inhibit the reduction of N_2 by rhizobia, limiting the activity of enzymes involved in this process and the development of root nodules [24]. Previous studies on the effects of nitrogen application on soybean nodulation and yield have involved the use of N application rates up to 100 kg per ha [20,23–27].

Given the above, the aim of this study was to determine the effect of varying nitrogen application rates on the number and weight of root nodules, the weights of the separated parts of the plant, and the content and uptake of N at the full flowering and ripe stages for soybeans fertilized at rates from 0 to $180\text{ kg N}\cdot\text{ha}^{-1}$. Based on the number of nodules formed, the potential of symbiotic bacteria to bind N_2 in the process of biological reduction was estimated.

2. Materials and Methods

2.1. Field Experiment Design

The field study was carried out in one growing season in 2018 in Siedlce, eastern Poland (GPS, N: 52°17'35.9", E: 22°30'51.3", 151 m a.s.l.). Soybeans (*Glycine max* (L.) Merr.) of the 'SG ANSER' cultivar were grown on experimental plots with an area of 2.25 m² (1.5 × 1.5 m). The actual experimental plot had an area of 1 m² (1 × 1 m), with a surrounding 25 cm buffer zone on which the test plant was grown at the same density, using the same fertilizer as in the inner part. The soil on which the experiment was carried out consisted of the following fractions: 2.0–0.05 mm = 86.3%; 0.05–0.002 mm = 9.5%; <0.002 mm = 4.2%. Selected properties of soil used in experiment are given in Table 1.

Table 1. Some properties of soil (0.30 m) used in experiment.

Soil Properties	Unit	Value
pH ₁ mol·dm ^{−3} KCl	-	5.8
N _{total}		0.60
C _{organic}		10.40
P _{total}		0.400
K _{total}	g·kg ^{−1}	0.349
Ca _{total}		0.567
Mg _{total}		0.141
S _{total}		0.095
P _{Egner-Riehm}		32.6
K _{Egner-Riehm}		48.4
Mg _{Schachtschabel}		20.2
B _{total}	mg·kg ^{−1}	1.82
Mn _{total}		21.8
Cu _{total}		4.50
Zn _{total}		16.50
Fe _{total}		2536

The experimental design included two factors. The first was the nitrogen (N) application rate: N0, 0 kg N·ha^{−1}, control; N1, 60 kg N·ha^{−1} (6 g N·m^{−2}); N2, 120 kg N·ha^{−1} (12 g N·m^{−2}); and N3—180 kg N·ha^{−1} (18 g N·m^{−2}). The second factor was the growth stage (B) in which the soybean plants were harvested and measurements were taken: B1, full flowering (BBCH 65); and B2, fully ripe (BBCH 90). The experiment was set up with a randomized block design in triplicate.

The forecrop for soybeans was spring triticale. After its harvest, plowing was carried out in the fall. In spring, the soil was loosened with a cultivator, leveled with a rake, plots were marked, and mineral fertilizers were applied. Mineral nitrogen was applied to the soil manually one day before sowing in the form of ammonium sulfate [(NH₄)₂SO₄]. This nitrogen fertilizer was chosen because it introduced sulfur into the soil, which has a beneficial effect on nitrogen metabolism in plants. Phosphorus and potassium were also applied one day before sowing at the same rate for all treatments: 45 kg P·ha^{−1} (4.5 g P·m^{−2}) as calcium hydrogen phosphate [Ca(H₂PO₄)₂] and 120 kg K·ha^{−1} (12 g K·m^{−2}) as potassium chloride (KCl). Separately weighed portions of fertilizer were applied manually to each plot. Sowing was carried out on 2 May at a density of 202 seeds per 2.25 m² (90 seeds per 1 m²). The seeds were sown manually, with a row spacing of 0.20 m, to a depth of 3–4 cm. The seeds were previously inoculated with a dressing containing symbiotic bacteria of the species *Bradyrhizobium japonicum*. The smallest number of plants that emerged per 1 m² among all the tested plots was 78. Therefore, 78 soybean plants were left on all remaining 1 m² plots, and the excess plants were removed (were torn out and left in the soil) so that different plant densities did not affect the results. Weeds were removed manually.

Monthly rainfall totals and average air temperatures during the soybean growing period are presented in Figure 1. The data were used to calculate Selyaninov's hydrothermal

index (k), which indicated that May and August were very dry, September was dry, and the conditions in May and July were optimal [28].

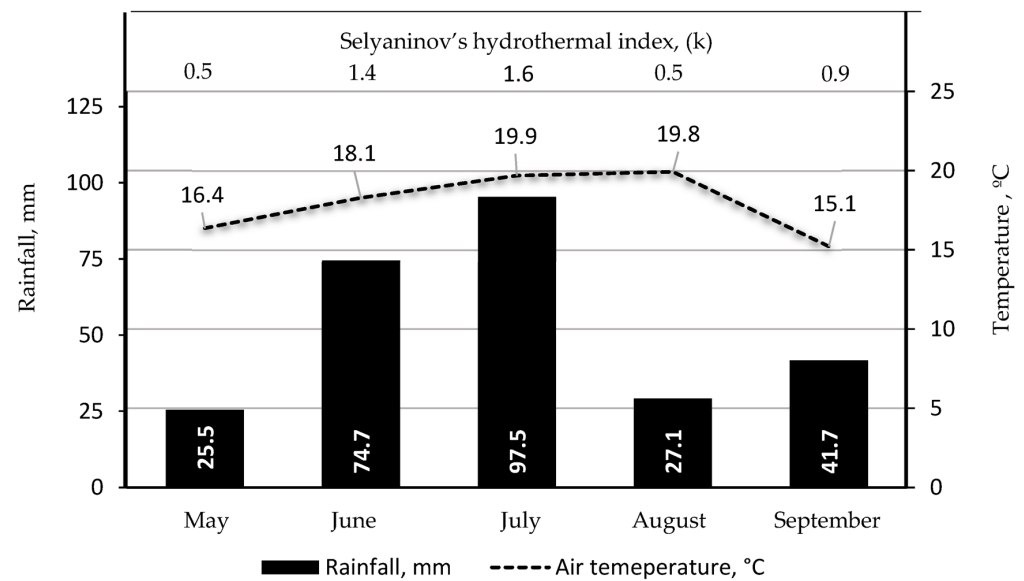


Figure 1. Cumulative monthly rainfall and average monthly air temperatures during the soybean growing season (May to September 2018, Institute of Meteorology and Water Management, National Research Institute in Warsaw) and values of the Selyaninov hydrothermal index (k) and moisture characteristics (w_m) of individual months: $k \leq 0.4$ —extremely dry; $0.4 < k \leq 0.7$ —very dry; $0.7 < k \leq 1.0$ —dry; $1.0 < k \leq 1.3$ —quite dry; $1.3 < k \leq 1.6$ —optimum; $1.6 < k \leq 2.0$ —moderately wet; $2.0 < k \leq 2.5$ —wet; $2.5 < k \leq 3.0$ —very wet; $k > 3.0$ —extremely wet.

2.2. Data Sampling and Measurements

The plants were harvested manually using a spade to dig them out of the soil at a depth of 30 cm, separately from each plot. Plants were collected from the inner part of each plot within an area of 1 m² (1 × 1 m). Harvesting was carried out on 25 July during the flowering stage and on 26 September at the fully ripe stage. The plants collected at BBCH stage 65 were separated into the roots, nodules, stems and leaves (due to the small weight of the flowers, they were included with the leaves). At BBCH stage 90, the nodules, roots, stems, leaves, pod husks, and seeds were separated.

The following parameters were determined in each plant sample obtained from 1 m²:

- Dry weight (DW) of separated parts dried at 105 °C;
- Number of root nodules, separately for each plant, which was used to estimate the potential of symbiotic bacteria to reduce N₂ to forms available for plants, according to Unkovich et al. [29];
- Total nitrogen content in the separated parts (nodules were included with the roots), determined by Kjeldahl's method [30].

2.3. Calculations and Statistical Analysis

Selyaninov's hydrothermal index (k) was calculated by the formula:

$$k = P/0.1\Sigma t, \quad (1)$$

where:

P —the monthly sum of precipitation in mm;

Σt —the monthly sum of air temperatures > 0 °C.

Nitrogen uptake by soybean plants (N_{up} , kg N·ha^{−1}) was calculated according to the following formula:

$$N_{up} = Y_{soybean} \cdot NC_{soybean} \quad (2)$$

where:

$Y_{soybean}$ —obtained dry mass of soybean plants (part of soybean plants, respectively);

$NC_{soybean}$ —total nitrogen content in soybean's plants dry mass (in separated organs, respectively).

Statistical analysis of the results was carried out using analysis of variance. The significance of the effect of the experimental factors on the values of individual traits was determined using the Fisher–Snedecor F-test, and the least significant difference values ($LSD_{0.05}$) for detailed comparison of means were calculated using Tukey's test at $p \leq 0.05$. In addition, the standard deviation (SD) and coefficient of variation (V) were calculated for selected parameters of soybean.

3. Results

Different nitrogen fertilizer application rates significantly influenced the dry weight of the roots, nodules, stems, seeds, and entire soybean plant (Table 2).

Table 2. Dry weight (mean values and $\pm$ SD) of different soybean plant parts (kg·ha^{−1}) as affected by N application level and growth stage in Siedlce, eastern Poland.

Growth Stages	N Doses kg·ha ^{−1}	Parts of Plant						Whole Mass
		Roots	Root Nodules	Stems	Leaves	Threshed Pods	Seeds	
BBCH 65 (B1)	0	711 ($\pm$ 57)	46 b ($\pm$ 11)	1069 ($\pm$ 215)	2232 ($\pm$ 363)			4058 ($\pm$ 637)
	60	782 ($\pm$ 40)	23 ab ($\pm$ 8)	1399 ($\pm$ 208)	2309 ($\pm$ 271)			4513 ($\pm$ 517)
	120	764 ($\pm$ 60)	07 a ($\pm$ 1)	1292 ($\pm$ 106)	2289 ($\pm$ 243)			4352 ($\pm$ 401)
	180	658 ($\pm$ 47)	02 a ($\pm$ 0)	1317 ($\pm$ 49)	2417 ($\pm$ 122)			4394 ($\pm$ 218)
BBCH 90 (B2)	0	727 ($\pm$ 42)	109 c ($\pm$ 26)	1986 ($\pm$ 165)	1704 ($\pm$ 115)	1777 ($\pm$ 63)	3352 b ($\pm$ 129)	9655 ab ($\pm$ 466)
	60	856 ($\pm$ 36)	110 c ($\pm$ 14)	2784 ($\pm$ 95)	2054 ($\pm$ 105)	1872 ($\pm$ 157)	3518 b ($\pm$ 167)	11,195 c ($\pm$ 553)
	120	694 ($\pm$ 36)	63 b ($\pm$ 10)	2574 ($\pm$ 123)	1969 ($\pm$ 177)	1759 ($\pm$ 93)	3318 b ($\pm$ 150)	10,377 bc ($\pm$ 318)
	180	631 ($\pm$ 41)	22 a ($\pm$ 9)	2276 ($\pm$ 209)	1856 ($\pm$ 204)	1637 ($\pm$ 103)	2826 a ($\pm$ 130)	9248 a ($\pm$ 254)
Means	Growth stages	B1	729 ($\pm$ 67)	19 a ($\pm$ 19)	1269 a ($\pm$ 188)	2312 b ($\pm$ 236)		4329 a ($\pm$ 437)
		B2	727 ($\pm$ 92)	76 b ($\pm$ 40)	2405 b ($\pm$ 342)	1896 a ($\pm$ 191)	1761 ($\pm$ 128)	10,119 b ($\pm$ 851)
	N doses	0	719 b ($\pm$ 46)	77 c ($\pm$ 39)	1527 a ($\pm$ 531)	1968 ($\pm$ 376)		6856 a ($\pm$ 3106)
		60	819 c ($\pm$ 53)	67 c ($\pm$ 49)	2092 c ($\pm$ 772)	2181 ($\pm$ 231)		7854 b ($\pm$ 3691)
		120	729 b ($\pm$ 59)	35 b ($\pm$ 31)	1933 bc ($\pm$ 709)	2129 ($\pm$ 258)		7365 ab ($\pm$ 3316)
		180	644 a ($\pm$ 42)	12 a ($\pm$ 13)	1796 b ($\pm$ 542)	2137 ($\pm$ 342)		6821 a ($\pm$ 2667)
	LSD _{0.05}	Growth stages (B)	n.s.	11	139	193		403
		N doses (A)	70	21	267	n.s.	468	772
		Interaction A/B	n.s.	30	n.s.	n.s.		1092

a–c—different lower-case letters in the columns indicate a significant difference of the mean values; n.s.—not significant.

The highest root weight was obtained following the application of 60 kg N·ha^{−1} (Table 2). The highest weight of nodules was produced by soybeans that were not fertilized with nitrogen and at the lowest application rate (60 kg N·ha^{−1}). In addition, in the fertilizer treatments, seed yield was higher with the application of 120 kg N·ha^{−1} than following the application of 180 kg N·ha^{−1}. The weights of stems and entire soybean plants were highest following the application of 60 and 120 kg N·ha^{−1} (Table 2). The statistical analysis showed an interaction between the nitrogen application rate and the growth stage for the weights of the nodules and the whole plant (Table 2). At BBCH stage 65, without nitrogen or adding 60 kg N ha^{−1}, the dry weights of root nodules significantly increased compared to higher N fertilizer doses. Moreover, the dry weights of whole plants remained unchanged irrespective of the nitrogen application rate. At BBCH stage 90, the weights of soybean nodules were highest for treatments without nitrogen application and at the lowest application rate of N (60 kg N·ha^{−1}). The lowest weight for root nodules was obtained after the application of 180 kg N·ha^{−1}. The whole weights of plants collected at BBCH

stage 90 were the highest following the application of the smallest amount of nitrogen ($60 \text{ kg} \cdot \text{ha}^{-1}$), and the lowest following the application of N at the highest rate ($180 \text{ kg} \cdot \text{ha}^{-1}$). The nitrogen application rate was not shown to significantly influence the weight of the soybean leaves or pod husks. The effect of the growth stage on the weight of the roots was also not found to be significant. The weights of the nodules, stems, and whole plants were higher at BBCH stage 90 than at BBCH stage 65, while the reverse pattern was observed for the leaves.

The number of nodules, calculated as the average per soybean plant harvested during the flowering stage, did not differ significantly in the control treatment without nitrogen application and following the application of $60 \text{ kg N} \cdot \text{ha}^{-1}$ (Figure 2). In comparison with these treatments, fewer nodules were found on the roots of soybeans fertilized with 120 and $180 \text{ kg N} \cdot \text{ha}^{-1}$. At the BBCH stage 90 and on average for both stages, soybeans without nitrogen application formed the most nodules. At this stage and on average for both stages, increasing the application of nitrogen caused a significant systematic decrease in the number of nodules formed. The coefficients of variation (V) reveal moderate variation in the number of nodules on the roots of soybeans grown without nitrogen application and following the application of 60 and $120 \text{ kg N} \cdot \text{ha}^{-1}$, and high variation following the application of $180 \text{ kg N} \cdot \text{ha}^{-1}$. Soybeans harvested at BBCH stage 90 had formed more than twice as many nodules than those at BBCH stage 65.

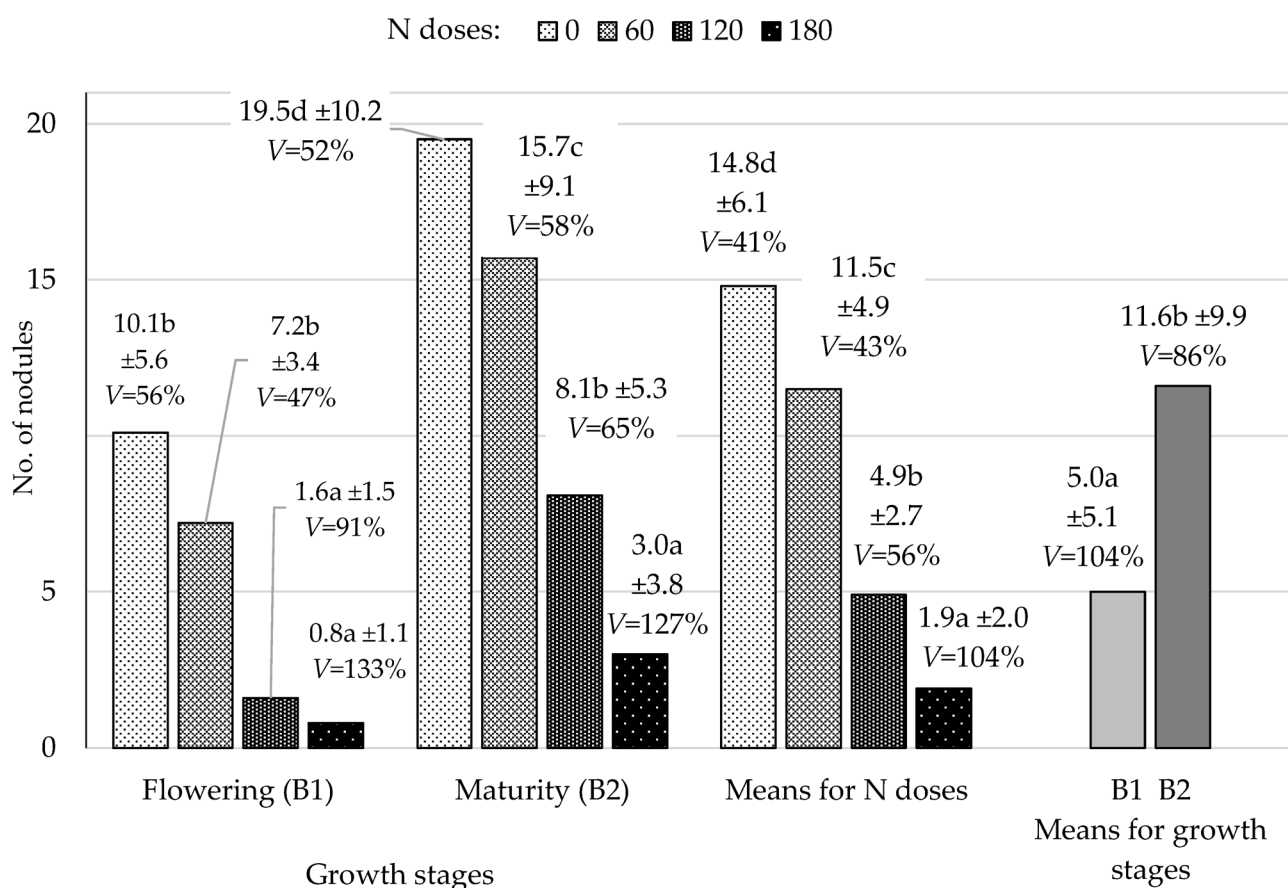


Figure 2. The effect of N fertilizer doses on the number of nodules in soybean plants (pieces per 1 plant). Values within the same growth stages, with the mean for N doses and mean for growth stages with different letters, where they are significantly different at $p < 0.05$ (mean values $\pm$ SD and variation coefficient V).

Nitrogen contents in the roots, stems, and leaves, and on average for the entire plant, were significantly dependent on the growth stage of the soybean plant (Table 3). The separated soybean organs contained more nitrogen at BBCH stage 65 than BBCH stage 90.

However, due to the high nitrogen content of the seeds, its average content in the entire plant was greater at BBCH stage 90 than BBCH stage 65. The statistical analysis showed no significant effect of the nitrogen application rate on N content in the roots, pod husks, seeds, or entire soybean plant. Its content in the stems and leaves was higher following the application of 180 kg N·ha^{−1} than in the treatments with 0, 60, and 120 kg N·ha^{−1}. In analyzing the occurrence of interactions, it should be emphasized that the nitrogen content in the leaves followed this pattern during the flowering stage of soybeans, while, at the fully ripe stage, it was not significantly dependent on the nitrogen application rate. Among all parts of the soybean plant, the seeds contained the most nitrogen. Conversion of nitrogen content to crude protein ($\times 6.25$) resulted in values of 30.7–35.8% (33.3% average).

Table 3. Nitrogen content (mean values $\pm$ SD) in soybean dry mass (g N·kg^{−1} D.M.) as affected by N application level and growth stage in Siedlce, eastern Poland.

Growth Stages	N Doses kg·ha ^{−1}	Parts of Plant					Weighted Averages in Whole Plant
		Roots with Nodules	Stems	Leaves	Threshed Pods	Seeds	
BBCH 65 (B1)	0	20.83 (± 2.08)	15.13 (± 1.66)	21.77 a (± 1.30)			19.85 (± 1.58)
	60	19.67 (± 1.94)	14.80 (± 0.56)	22.40 a (± 1.44)			19.57 (± 1.22)
	120	20.50 (± 1.75)	16.17 (± 1.85)	24.57 a (± 1.39)			21.34 (± 1.39)
	180	23.20 (± 2.19)	18.00 (± 1.45)	29.77 b (± 2.01)			21.25 (± 1.64)
BBCH 90 (B2)	0	14.57 (± 0.75)	8.97 (± 0.71)	16.43 (± 0.83)	4.93 (± 0.75)	57.23 (± 2.06)	26.79 b (± 1.24)
	60	13.63 (± 1.63)	8.10 (± 0.72)	16.20 (± 0.46)	5.03 (± 0.32)	54.40 (± 4.15)	24.11 ab (± 1.31)
	120	13.10 (± 1.04)	8.67 (± 0.64)	15.27 (± 1.46)	5.20 (± 0.44)	52.55 (± 1.91)	23.47 a (± 1.10)
	180	13.20 (± 1.31)	10.57 (± 1.06)	15.13 (± 0.61)	4.93 (± 0.31)	49.13 (± 2.20)	22.44 a (± 0.55)
Means	growth stages	B1	21.05 b (± 2.19)	16.03 b (± 1.81)	24.63 b (± 3.54)		21.51 a (± 2.68)
		B2	13.63 a (± 1.21)	9.08 a (± 1.17)	15.76 a (± 0.98)	5.03 (± 0.43)	24.20 b (± 1.92)
	N doses	0	17.70 (± 3.71)	12.05 a (± 3.57)	19.10 a (± 3.08)		23.32 (± 4.01)
		60	16.65 (± 3.67)	11.45 a (± 3.72)	19.30 a (± 3.53)		21.84 (± 2.73)
		120	16.80 (± 4.25)	12.42 a (± 4.29)	19.92 a (± 5.25)		22.41 (± 1.61)
		180	18.20 (± 5.71)	14.28 b (± 4.23)	22.45 b (± 8.13)		23.84 (± 1.89)
LSD _{0.05}	growth stages (B)	1.50	0.95	1.20			1.49
	N doses (A)	n.s.	1.82	2.30	n.s.	n.s.	n.s.
	Interaction A/B	n.s.	n.s.	3.52			n.s.

a, b—different lower-case letters in the columns indicate a significant difference of the means values; n.s.—not significant.

The amounts of nitrogen accumulated in the roots and leaves, and on average in the whole plant, were significantly dependent on the growth stage of soybean (Table 4). Soybeans accumulated more nitrogen in these organs at BBCH stage 65 than BBCH stage 90. However, due to the high accumulation of nitrogen in the seeds, the amount accumulated in the whole plant, on average, was 2.6 times higher at BBCH stage 90 as compared to the flowering stage. The nitrogen application rate was shown to have a significant influence on its accumulation in the whole plant as well as in its parts, except for the pod husks. The most nitrogen in the roots and whole plant was accumulated by soybeans fertilized with 60 kg N·ha^{−1}, with the least following the application of 180 kg N·ha^{−1} (Table 4). However, in the treatments with 0, 60 and 120 kg N·ha^{−1}, N accumulation did not differ significantly in the roots, leaves, seeds or entire plant. Interaction effects indicated that nitrogen accumulation in the leaves followed this pattern at BBCH stage 65, however, at BBCH stage 90, it was not significantly dependent on nitrogen application. In the flowering stage, accumulation of nitrogen in the entire soybean plant was higher following the application of 180 kg N·ha^{−1} than in the treatments with 0 and 60 kg N·ha^{−1}, while the relationship was reversed at BBCH stage 90. In the flowering stage, the roots (together with the nodules), stems, and leaves (with the flowers) accumulated 16.9%, 21.9%, and 61.2%, respectively, of the total accumulated nitrogen. In the fully ripe stage, the roots (with nodules), stems, leaves, pod husks and seeds accumulated 4.5%, 8.8%, 12.2%, 3.6% and 70.9%, respectively, of the total accumulated nitrogen.

Table 4. Nitrogen uptake (mean values $\pm$ SD) for soybeans ($\text{kg N}\cdot\text{ha}^{-1}$) as affected by the N application level and growth stage in Siedlce, eastern Poland.

Growth Stages	N Doses $\text{kg}\cdot\text{ha}^{-1}$	Parts of Plant					Whole Mass
		Roots with Nodules	Stems	Leaves	Threshed Pods	Seeds	
BBCH 65 (B1)	0	15.7 (± 0.3)	15.9 (± 1.4)	48.3 a (± 5.1)			79.9 a (± 6.3)
	60	15.8 (± 1.1)	20.6 (± 2.6)	51.5 a (± 3.0)			87.9 a (± 4.9)
	120	15.8 (± 1.0)	20.7 (± 0.8)	56.0 a (± 3.0)			92.5 ab (± 2.9)
	180	15.3 (± 2.3)	23.8 (± 2.8)	72.0 b (± 6.7)			111.1 b (± 11.4)
BBCH 90 (B2)	0	12.2 bc (± 0.8)	17.8 (± 1.8)	28.0 (± 2.7)	8.7 (± 1.1)	191.8 b (± 8.1)	258.5 bc (± 11.5)
	60	13.1 c (± 1.1)	22.6 (± 2.4)	33.3 (± 2.3)	9.5 (± 1.4)	190.9 b (± 6.9)	269.4 c (± 3.4)
	120	9.9 ab (± 0.4)	22.3 (± 0.8)	30.2 (± 5.4)	9.1 (± 0.8)	172.0 b (± 8.8)	243.5 b (± 11.9)
	180	8.6 a (± 0.5)	24.1 (± 4.1)	28.0 (± 2.0)	8.1 (± 0.9)	138.7 a (± 4.9)	207.5 a (± 9.0)
Means	Growth stages	B1	15.7 b (± 1.2)	20.3 (± 3.4)	56.9 b (± 10.3)		92.9 a (± 13.4)
		B2	10.9 a (± 2.0)	21.7 (± 3.3)	29.9 a (± 3.7)	8.9 (± 1.0)	244.7 b (± 25.7)
	N doses	0	13.9 ab (± 2.0)	16.9 a (± 1.8)	38.2 a (± 11.7)		169.2 ab (± 98.2)
		60	14.5 b (± 1.8)	21.6 b (± 2.5)	42.4 a (± 10.2)		178.7 b (± 99.4)
		120	12.8 ab (± 3.3)	21.5 b (± 1.1)	43.1 a (± 14.7)		168.0 ab (± 83.0)
		180	12.0 a (± 4.0)	23.9 b (± 3.1)	50.0 b (± 24.5)		159.3 a (± 53.6)
	LSD _{0.05}	Growth stages (B)	1.0	n.s.	3.6		7.8
		N doses (A)	1.9	4.0	6.8	n.s.	15.0
		Interaction A/B	2.7	n.s.	9.7		21.2

a–c—different lower-case letters in the columns indicate a significant difference of the means values; n.s.—not significant.

4. Discussion

Based on 207 experiments under various environmental conditions in the US, the effect of nitrogen fertilization did not cause significant changes in soybean yield at application rates of up to 100 kg ha^{-1} in 93% of cases [31]. A significant increase in yield was obtained in only a few cases [31]. The authors also showed that variation in the seed yields of soybeans were associated to the greatest extent (68%) with the effects of environmental conditions. The results of the present study indicate that nitrogen application at 60 and $120 \text{ kg N}\cdot\text{ha}^{-1}$ caused no significant changes in seed yield, and the highest application of N ($180 \text{ kg N}\cdot\text{ha}^{-1}$) decreased the weight of the seeds. Nitrogen application has been shown to increase soybean seed yield in sites with low soil fertility [32] and in favorable to high-yield conditions [27]. In the present study, soybeans were grown in soil with low fertility, so the high seed yields obtained in the control treatment (without N application) and following its lowest application ($60 \text{ kg}\cdot\text{ha}^{-1}$) were most likely due to the uptake of large amounts of nitrogen fixed from the atmosphere. Nitrogen application at 60 and $120 \text{ kg}\cdot\text{ha}^{-1}$ resulted in higher weight of the vegetative parts than in the control treatment, but the significance of the differences was not confirmed in all cases. Mrkowački et al. [26], in a study conducted under Mediterranean conditions, obtained the highest above-ground weight for soybeans at BBCH stage 65 following nitrogen application at the highest rates applied in their experiment (60 and $90 \text{ kg}\cdot\text{ha}^{-1}$), and in the fully ripe stage following the lowest application N rates ($30 \text{ kg N}\cdot\text{ha}^{-1}$).

The literature contains few studies on the effect of the application of large amounts of nitrogen, exceeding $150 \text{ kg}\cdot\text{ha}^{-1}$, on the yield of soybean grown in Central Europe; application rates usually have not exceeded $100 \text{ kg}\cdot\text{ha}^{-1}$ N [33–36]. Marin and Dumitru [37] reported that nitrogen rates up to $200 \text{ kg}\cdot\text{ha}^{-1}$ significantly increased soybean yield. Following the application of 150 and $200 \text{ kg}\cdot\text{ha}^{-1}$, these authors reported seed yields at levels of 334 and $468 \text{ kg}\cdot\text{ha}^{-1}$ respectively, indicating low nitrogen fertilizer use efficiency (2.23 and 2.34 kg of seeds per kg N respectively). In the present study, the application of $180 \text{ kg N}\cdot\text{ha}^{-1}$ did not produce positive results in the form of a higher seed yield or higher weight of the entire plant in comparison to the effect of lower application rates, or even the control without nitrogen fertilizer.

Increasing the amount of nitrogen fertilizer used reduced the number of root nodules formed. Application of 120 and $180 \text{ kg N}\cdot\text{ha}^{-1}$ had a particularly negative effect on this trait. This is in agreement with generally accepted opinions and studies conducted

in other parts of the world, in which a reduction in nodule number and weight has been observed following the application of nitrogen to the soil, even at rates lower than $100 \text{ kg} \cdot \text{ha}^{-1}$ [24–27].

Based on the number of root nodules formed, it is possible to estimate the potential of soybean rhizobia to reduce molecular nitrogen (N_2) to forms available for plants [29]. According to the criteria proposed by the authors, in the present study, the microorganisms living in symbiosis with soybeans, collected in the flowering stage from the treatments without nitrogen application and following the application of $60 \text{ kg N} \cdot \text{ha}^{-1}$, showed low potential to reduce N_2 . Of these treatments, there may have been too little available nitrogen from this source to meet the nutritional needs of the host plant. At this growth stage, following the application of 120 and $180 \text{ kg N} \cdot \text{ha}^{-1}$, the potential of bacteroids to bind nitrogen in the biological reduction process was very small. Based on the number of nodules in the fully ripe stage, the N_2 reduction potential of soybeans without nitrogen application was estimated to be very high. At this growth stage, symbiotic bacteria in soybeans fertilized with 60, 120, and $180 \text{ kg N} \cdot \text{ha}^{-1}$ had high, low, and very low N_2 reduction potential. This indicates that the potential of bacteria living in symbiosis with soybeans to bind atmospheric nitrogen in the biological reduction process decreases as the level of nitrogen application increases, which suggests that soybeans take up nitrogen from a more easily available source that requires the plant to incur energy costs. Given the above, as well as the weight of the soybeans obtained in the fertilized treatments, it can be concluded that high yield was not dependent on the level of nitrogen fertilization. High seed yields from this plant were obtained without nitrogen application and at up to $120 \text{ kg N} \cdot \text{ha}^{-1}$. Spasić et al. [38] reported higher seed yields without nitrogen application and reduced productivity of many soybean cultivars in conditions of increased nitrogen application. Barker and Sawyer [39] also showed that nitrogen application is not justified at the early reproductive stages of soybeans, as it only increases the nitrogen concentration in the plant, without affecting seed yield. Spasić et al. [38] reported that increased nitrogen application led to a minor increase in the crude protein content in the seeds. On average, from three years of cultivation, following application of 50, 100, and $150 \text{ kg N} \cdot \text{ha}^{-1}$, the authors obtained an increase in the content of crude protein in soybean seeds amounting to 0.07%, 0.10%, and 0.46%, respectively. They reported that nitrogen application caused a marked increase in this parameter in a year with an optimal rainfall distribution during the growing season. An increase in the content of crude protein in soybean seeds following the application of mineral nitrogen has also been reported by others [33,34]. Marin and Dumitru [37], in most cases, obtained the highest nitrogen content in soybean seeds following N application at $150 \text{ kg} \cdot \text{ha}^{-1}$.

In the present study, increasing the level of nitrogen applied in a single portion before sowing decreased the content of N (and crude protein, after multiplying by the conversion factor 6.25) in soybean seeds, but the differences were not statistically significant. These observations may be linked to the uptake of nitrogen from fertilizer by soybeans in the initial growth period, in combination with limitation of the production of root nodules and limitation of the uptake of N_2 from the atmosphere. In effect, at BBCH stage 65, the nitrogen content in the stems and leaves was increased by the increased application of mineral N. The systematic reduction in nodulation caused by increasing application of nitrogen, in combination with the gradual dispersion of N in the environment from the fertilizer applied before sowing, may have led to an insufficient amount of N available for plants after the flowering stage. This may have been the cause of the lower nitrogen accumulation in soybeans fertilized with N at the two highest rates (120 and $180 \text{ kg} \cdot \text{ha}^{-1}$) in comparison with the lowest rate ($60 \text{ kg} \cdot \text{ha}^{-1}$) and the control treatment ($0 \text{ kg} \cdot \text{ha}^{-1}$). The supply of nitrogen during seed filling supplements existing reserves, thereby preventing premature ageing of soybean plants and increasing seed yield [40]. If the amount of available nitrogen does not meet the requirements of the soybean plant during this period, the plant mobilizes N accumulated in the leaves to the seeds. In effect, this limits photosynthesis, thereby reducing the yield potential of the plant [41]. Szostak et al. [31],

Głowacka et al. [36], and Zainab et al. [42], in consideration of seed yield and chemical composition, have recommended applying part of the total amount of nitrogen fertilizer (one half to three quarters) before sowing and the rest during pod and seed development. This split application resulted in high seed yields where soybeans formed a small number of root nodules, while, in the case of intensive nodulation, application only before sowing was the most beneficial for yield [33]. The authors ascribe the increase in seed yield following application of nitrogen before sowing to increased activity of the root system, an increased leaf area index, and a faster rate of photosynthesis [43,44].

In the fully ripe stage, mainly 70.8% (from 66.8% on N180 treatment to 74.2% on N0 treatment) of the nitrogen taken up by soybean was accumulated in the seeds, so just under 30% returned to the soil. In absolute values, this was 176.3 kgN·ha⁻¹ (seeds) and 71.4 kgN·ha⁻¹ (crop residue). The nitrogen harvest index (NHI) in soybeans cultivated in the conditions of Central and Eastern Europe range from 50% to 70% [45].

5. Conclusions

Increasing pre-sowing application of mineral nitrogen did not increase the seed yield or mass for threshed pods, leaves and roots of soybean plants, while it increased the mass of stems and reduced the number and weight of root nodules formed in the full flowering and full maturity stages. This indicates a limited potential for soybean plants regarding the biological reduction of N₂ and its uptake from the atmosphere. The number of root nodules increased from the flowering stage to the full maturity stage, indicating an increased potential for atmospheric nitrogen reduction at this time. Varied levels of nitrogen application in the range of 0–180 kgN·ha⁻¹ did not influence the content of N in the seeds, threshed pods, roots, or whole soybean plants. Only the highest dose of nitrogen (180 kgN·ha⁻¹) increased the nitrogen content in stems and leaves—the vegetative organs of soybean. Accumulation of nitrogen in the seeds and whole soybean plant did not vary following the application of nitrogen in the range of 0–120 kg N·ha⁻¹ but decreased following the application of 180 kg N·ha⁻¹. The amount of nitrogen accumulated in the stems increased after applying all nitrogen doses (60, 120, and 180 kgN·ha⁻¹), while only in the leaves after applying the highest dose (180 kgN·ha⁻¹). The results indicate that the use of a high pre-sowing nitrogen application rate for soybean plants is not justified, as it did not increase seed yield or the content and accumulation of N in the seeds (and thus the content and yield of crude protein, which is important for human food and in feed for animals). In return, this may lead to high savings in energy costs for additional fertilizer production, and reducing nitrogen fertilizer application could be regarded as a climate change mitigation strategy.

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