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The Effect of Nitrogen and Sulphur Application on Soybean Productivity Traits in Temperate Climates Conditions

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Abstract: Both nitrogen and sulphur are important macronutrients necessary for the proper development and yield of soybean. Moreover, sulphur plays a special role in nitrogen metabolism in the plant, and sulphur deficiency leads to a reduction in the utilization of nitrogen from fertilizer. The objective of this study was to assess the effect of nitrogen and sulphur application on the yield and quality traits of soybean seeds. The following factors were analyzed in the experiment: I. Nitrogen application rate: 0, 30 and 60 kg ha^{-1} applied at different times (before sowing and/or at the start of the seed filling stage); II. Sulphur application rate: 0 and 40 kg ha^{-1} applied in two portions: half during the development of lateral shoots and half at the start of flowering. Thus the 14 fertilizer combinations were obtained. Result show that the highest seeds yield was obtained in the combinations with 60 kg N applied ½ before sowing + ½ after emergence (BBCH 73-75) and ¾ before sowing + ¼ after emergence. In these combinations, sulphur did not significantly affect seed yield. In the remaining nitrogen application, sulphur application significantly increased the seed yield. Taking into account the yield and the chemical composition of the soybean seeds, fertilization with 60 kg N ha^{-1} in two portions can be recommended—½ or ¾ before sowing and the remainder during the development of pods and seeds—in combination with sulphur application.

Keywords: nitrogen fertilization; sulphur; crude fat; crud protein; seeds yield; potassium; magnesium; phosphorus

Citation: Głowacka, A.; Jariene, E.; Flis-Olszewska, E.; Kiełtyka-Dadasiewicz, A. The Effect of Nitrogen and Sulphur Application on Soybean Productivity Traits in Temperate Climates Conditions. *Agronomy* **2023**, *13*, 780. https://doi.org/10.3390/agronomy13030780

Academic Editors: Gang Li, Dong-Xing Guan and Daniel Menezes-Blackburn

Received: 9 February 2023
Revised: 2 March 2023
Accepted: 6 March 2023
Published: 8 March 2023

1. Introduction

Soybean is one of the most important crop plants in the world. As it takes up fairly large amounts of essential nutrients, it should be grown on a site with adequate nutrient content [1–3]. One essential element for the growth and development of soybean is nitrogen. Soybean requires a large amount of nitrogen due to the high protein content in the seeds—about 35–40%. Like other legumes, soybean takes up nitrogen from two sources: the atmosphere and mineral fertilizers [4]. Atmospheric nitrogen is fixed by rhizobia. Nitrogen fertilization for legume plants is usually limited to application of starter fertilizer. The level of application varies depending on soil conditions but is usually much lower than standard levels of this nutrient used to fertilize other plants. However, according to many authors, biological nitrogen fixation meets only about 50–60% of soybean's demand for nitrogen, resulting in 80–90% of the maximum yield obtained in the case of adequate nitrogen fertilization [5,6]. Nitrogen application mainly affects crop yield. According to Lorenc-Kozik and Pisulewska [7], a nitrogen application rate that beneficially influences soybean yield varies between 30 kg N ha^{-1} and 60 kg N ha^{-1}. Fertilization of soybean with nitrogen has a beneficial effect on elements of the yield structure such as plant height, pod number per plant, and seed weight per plant. Nitrogen application can also modify the chemical composition of the seeds. Lower levels increase their fat content, while higher levels increase the content of protein [8–10]. In the case of soybean fertilization with nitrogen, not only the level of application is important, but the time of application as well [8,11]. Starter nitrogen fertilizer applied before sowing is aimed at supplying easily available nitrogen from the soil during seedling development and has been shown by numerous studies to increase soybean seed yield [7–9,12] According to [13] soybean has a relatively high demand for N during the seed-filling stage, and biological fixation of N and a low level of starter fertilizer may not supply an adequate amount of N for full exploitation of the plant's yield potential. Therefore, N application during generative growth can increase crop yields.

Another essential nutrient for the normal functioning of plants is sulphur, which is a component of vitamins and amino acids, and thus of proteins. The presence of sulphur is essential for biosynthesis of protein in the seeds. It positively affects not only the quantity but also the quality of crude protein in the harvested crop, especially in the case of legumes [14,15]. Plants of the *Fabaceae* family have a moderate demand for this nutrient [16]. For legumes, depending on the species and climate-soil conditions, the recommended application rate ranges from 20 to 60 kg S ha^{-1} [14,17,18]. It should be remembered, however, that a single portion of this nutrient should not exceed 20 kg S ha^{-1} [14,19]. Many studies [14,16,17,20] indicate a strong interaction between sulphur and nitrogen as essential nutrients for synthesis of amino acids making up proteins. Sulphur plays a special role in nitrogen metabolism in the plant, and sulphur deficiency leads to a reduction in the utilization of nitrogen from fertilizer. Sulphur is an activator of processes regulating C and N metabolism in the plant, and in this way increases the rate of transformation of nitrogen taken up by the plant into protein. Plants that are well supplied with nitrogen and sulphur increase the amount of nitrogen incorporated into organic structures. Sulphur also takes part in fixation of atmospheric nitrogen by rhizobia and in reduction in nitrates to ammonia. Many authors have confirmed the positive effect of sulphur application on fixation of atmospheric nitrogen by the root nodules of legumes and on utilization of mineral nitrogen, and thus on production of plant biomass [16,20,21]. Therefore, adequate supply of these nutrients to plants enables full exploitation of their yield potential. In the absence of sulphur, they produce protein with much lower content of sulphur-containing amino acids, especially methionine, which is one of the most valuable amino acids determining the nutritional value of plants [22–24]. The effectiveness of sulfur fertilization depends on many factors, including the dose and date of application. According to many authors [25,26], oilseeds have a high demand for sulfur, especially from the budding phase to the formation of siliques. The availability of sulfur during this period ensures the proper growth and development of rapeseed. The study conducted by Barczak et al. [19] confirmed that the foliar sulphur application (in the phase of not completely covered interrows until full flowering), as compared with the soil application, showed a better effect on the seed and straw yield size.

Research results confirm the beneficial effect of sulphur on the yield of legume plants, such as narrow-leaved lupin [19], field bean [27], common bean and broad bean [22,24]. Other authors have also confirmed positive effects of legume fertilization with sulphur on the content and uptake of nitrogen. The effect of nitrogen application on the yield and quality of soybean crops is also a frequent subject of research [14,28–30]. Most of the research on the reaction of soybean to nitrogen and sulfur fertilization was conducted in countries with a warm climate, i.e., Iran [31,32] India [33–37]. There are few studies conducted in temperate climate conditions especially with newer not genetically modified cultivars—on the effect of nitrogen and sulphur application on the yield and chemical composition of soybean seeds [12]. In view of the above, a study was carried out to test the effect of nitrogen and sulphur application on the yield of soybean and the content of protein, fat and selected macroelements in the seeds. The main objective of the research was to determine how much (and when) N and S fertilization should be used to obtain Ethe best production characteristics in soybean cultivation in the climatic conditions of south-eastern Poland.

2. Materials and Methods

2.1. Site Description, Experimental Design and Field Management

A 3-year field experiment was carried out in 2015–2017 under rainfed conditions on a private farm in Zamość District, in south-eastern Poland (50°43′34″ N, 23°39′11″ E).

The experimental field was located on soil with the granulometric composition of clayey silt, slightly acidic, with moderate content of phosphorus and potassium (Table 1). The conventional (not genetically modified) soybean cultivar Amandine, a medium-early cultivar (maturity group MG 000) with high yield potential, was planted in the experiment.

Table 1. Selected soil properties (0–30 cm depth) in experimental field.

Soil Characteristic	Value
Texture class [1]	Clayey silt
Sand (2–0.05 mm)	21%
Silt (0.05–0.002)	70%
Clay (<0.002)	9%
pH_{KCl}	6.6
Total nitrogen (g kg^{-1})	1.3
Organic carbon (g kg^{-1})	19.1
Available forms (mg kg^{-1})	
Phosphorus	175
Potassium	201
Magnesium	54
Copper	43.2
Manganese	187.2
Iron	942
Zinc	9.6

[1] According to [38].

The following factors were analyzed in the experiment:

I. Nitrogen application rate: 0, 30 and 60 kg ha^{-1} applied at different times (before sowing and/or at the start of the seed filling stage BBCH 73–75)

II. Sulphur application rate: 0 and 40 kg ha^{-1} applied in two portions: half during the development of lateral shoots (BBCH 209–210) and half at the start of flowering (BBCH 60–61)

The phonological stages (BBCH) of soybean were encoding according to [39]. The nitrogen dose resulted from fertilizer recommendations for legumes in Poland and previous own research [8]. The sulphur dose was determined based on the recommendation of other authors [14,18,22].

Thus, the following 14 fertilizer combinations were obtained (Table 2).

The experiment was set up in a randomized split-plot design in three replicates. The area of the plots was 12.5 m^2 (2.5 m × 5 m). Nitrogen application rates has been designated as the main plot and sulphur application rate as the subplot.

Soybean was sown between 30 April and 2 May, at a rate of 70 seeds m^{-2}, and the row spacing was 20 cm. Soybean seeds were prepared for sowing in FIX FERTIG technology. In this process the seeds are coated with rhizobia together with a polymer, which acts as a preservative and also protects against solar radiation. Phosphorus (in the form of Fos Dar fertilizer) and potassium (60% potassium chloride) were applied once before sowing, at the same rates in all treatments: P—21.1 kg ha^{-1}, K—76.4 kg ha^{-1}. Nitrogen fertilizer was applied in the form of 34% ammonium nitrate, and sulphur in the form of magnesium sulphate heptahydrate. In each year of the study the precursor crop for soybean was winter wheat. After harvesting of the precursor crop, skimming was carried out (shallow ploughing at 8–10 cm), followed by harrowing and pre-winter ploughing to a medium depth (22–25 cm). Harrowing was carried out in early spring, followed by pre-sowing tillage with a tillage machine equipped with a roller. Sowing was carried out using a mechanical seeder for cereals. Plant protection was limited to chemical weed control. Directly after sowing herbicides were applied to the soil: Sencor Liquid 600 SC (biologically active substance—metribuzin) in the amount of 0.5 dm^3 ha^{-1} and Dual Gold 960 EC (biologically active substance—S-metolachlor) in the amount of 1.0 dm^3 ha^{-1}. Soybean was harvested with a combine at full maturity (BBCH 99) in the middle 10 days of September.

Table 2. Researched fertilizer combinations.

Fertilizer Combination	Dose and Time of Application	
	Nitrogen	Sulphur
N0-S0	without nitrogen	without sulphur
N0-S40	without nitrogen	40 kg S ha^{-1}
N30 (30:0)-S0	30 kg N ha^{-1} before sowing	without sulphur
N30 (30:0)-S40	30 kg N ha^{-1} before sowing	40 kg S ha^{-1}
N30 (15:15)-S0	15 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling	without sulphur
N30 (15:15)-S40	15 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling	40 kg S ha^{-1}
N30 (0:30)-S0	30 kg N ha^{-1} at the start of the seed filling	without sulphur
N30 (0:30)-S40	30 kg N ha^{-1} at the start of the seed filling	40 kg S ha^{-1}
N60 (15:45)-S0	15 kg N ha^{-1} before sowing + 45 kg N ha^{-1} at the start of the seed filling	without sulphur
N60 (15:45)-S40	15 kg N ha^{-1} before sowing + 45 kg N ha^{-1} at the start of the seed filling	40 kg S ha^{-1}
N60 (30:30)-S0	30 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling	without sulphur
N60 (30:30)-S40	30 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling	40 kg S ha^{-1}
N60 (45:15-S0	45 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling	without sulphur
N60 (45:15)-S40	45 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling	40 kg S ha^{-1}

2.2. Features of the Yield Components and Laboratory Analysis

After harvest, at the full maturity stage (BBCH 99) of entire plot with a combine, the seed yield was determined and expressed per hectare for a moisture level of 15%. After harvesting, seed samples (0.5 kg) were taken from every plot for chemical analysis. Prior to harvest of soybean, 10 plants were randomly selected from each plot to determine elements of the yield components: plant height, pod number per plant, seed number per pod, and thousand seed weight.

The soybean seeds were analyzed for:

- crude protein by Kjeldahl's method (according to CLA/PSO/13),
- crude fat by the Soxhlet extraction-weighing method (according to CLA/PSO/10);
- total sulphur by the Bradley–Lancaster nephelometric method (following wet mineralization using concentrated sulphuric acid with 30% perhydrol),
- phosphorus by spectrophotometry (according to CLA/PLC/28),
- potassium, magnesium and calcium by Atomic Absorption Spectrometry with excitation in the air-acetylene flame (according to CLA/ASA/2).

The analyses were carried out at the Central Laboratory of Agroecology of the University of Life Sciences in Lublin. The total protein content in the seeds was calculated as 6.26 × total N.

2.3. Statistical Analysis

Statistical analysis of the results was performed by analysis of variance (ANOVA) in STATISTICA 13 PL software (Tulsa, OK, USA). Three-way ANOVA was carried out to determine the effect of the year, nitrogen fertilization, and sulphur fertilization on soybean yield, its components, and the chemical composition of the seeds. The effect of year, nitrogen fertilization, cultivar, and their interactions were analyzed using a split–split-plot design with the year being designed as whole plots, nitrogen fertilization as subplots, and sulphur fertilization as sub–subplots. The differences between means were determined using Tukey's post-hoc test at $p < 0.05$. As no significant interaction of the factors and years of the study was confirmed, the results presented are averages from three years. The effect of the interaction of factors is presented. If the interaction of factors was not significant, the main effect was presented.

3. Results

3.1. Meteorological Conditions

The experiment was carried out under rainfed conditions The weather conditions during the study period are presented in Figure 1. Meteorological data come from the Lublin-Felin automatic meteorological station, located on the premises of the Department of Plant Production Technology and Commodity Science of the University of Life Sciences in Lublin. The station is located approximately 90 km from the site of the field experiment the meteorological data were used to calculate Selyaninov's hydrothermal coefficient (Figure 2) according to the following formula: $k = (p \times 10)/\Sigma t$ where p is the sum of ten-day monthly precipitation (mm) and Σt is the sum of average daily temperatures from a tenday period/month (°C). Ranges of values for the coefficients were designated according to the scale developed by Skowera et al. [40].

In 2015 and 2016 the precipitation totals in the period from April to September were similar (330–335 mm) and higher than the long-term average. In 2017 the precipitation total during the growing season was about 358 mm, which was close to the long-term average. Particularly heavy rainfall was noted in May and April 2015 and in July 2016 and 2017. In 2015 very low rainfall was noted in June and in August.

Selyaninov's coefficient (Figure 2) indicated that the period from May to June in 2015 and 2016 was dry or very dry. In 2017 highly unfavorable temperature and moisture conditions were noted in the period from June to August.

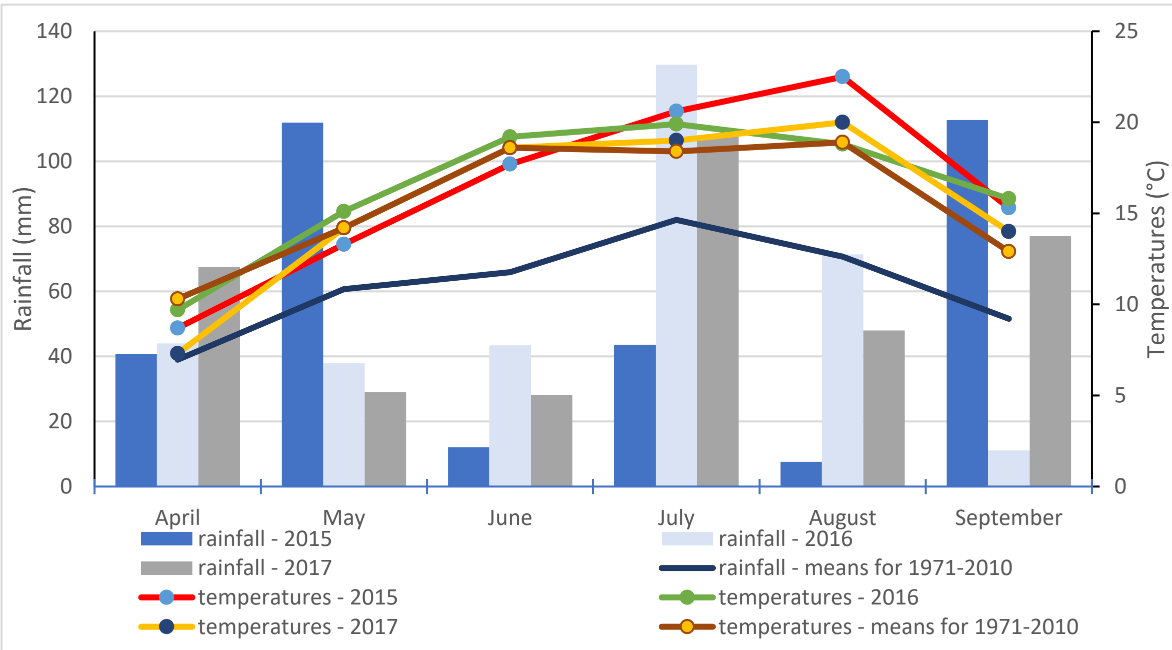

Figure 1. Air temperature (each point represents the average temperature of each month) and rainfall distribution during the growing period of April–September as compared to the long-term means (1971–2010).

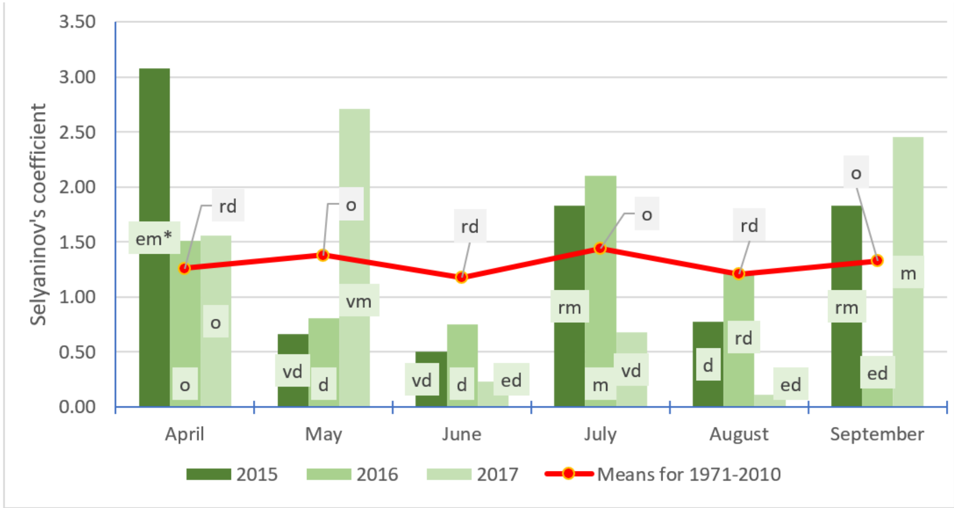

Figure 2. Selyaninov's coefficient during the growing period as compared to the long-term means (1971–2010). * Interpretation of the value of the Sielianinov coefficient according to [40]: ed—extremely dry, vd—very dry, d—dry, rd—rather dry, o—optimal, rm—rather moist; m—moist, vm—very moist, em—extremely moist.

3.2. Yield and Elements of the Yield Structure

The lowest yield of soybeans seeds, at a level of 20.9 dt ha^{-1}, was obtained from the control treatment, in which no fertilizer was applied (Figure 3). Fertilization with nitrogen and sulphur significantly increased the yield of soybean seeds. The most beneficial effect was obtained in the treatments in which 60 kg N was applied 1/2 before sowing +1/2 after emergence and 3/4 before sowing +1/4 after emergence. In these combinations, the seed yield was 8–10 dt ha^{-1} higher than in the combination without fertilizer, while sulphur application had no significant effect on yield. In the remaining treatments, sulphur application significantly increased the yield of soybean seeds (Figure 3). It should be noted that the greatest differences were noted when sulfur was applied in the combination without nitrogen fertilizer, were the yield of soybean seed increased by 18%. In the combinations with 30 kg N ha^{-1}, sulphur application increased yield by 6–7%.

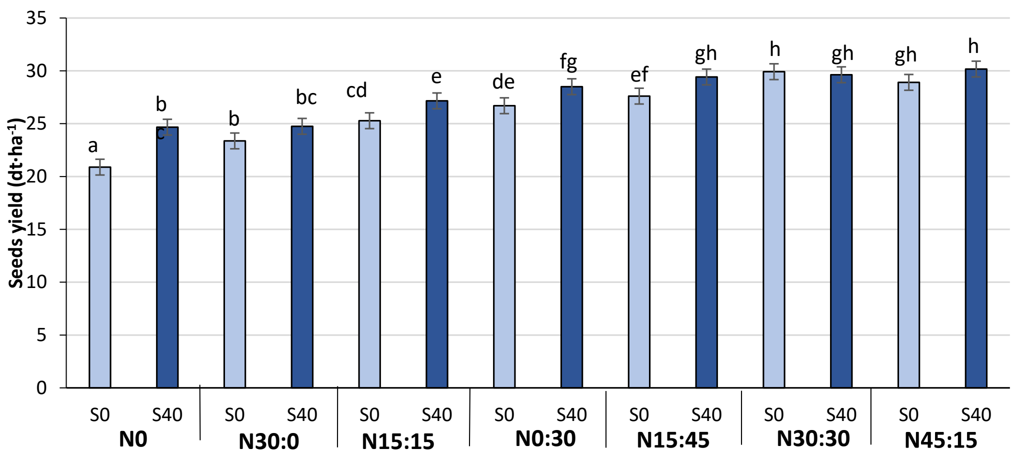

Figure 3. Effects of nitrogen × sulphur application on the yield of soybean seeds. Means marked with the same letter do not differ significantly at p = 0.05, bars mean SE. S0—without sulfur, S40—40 kg S ha^{-1}, N0—without nitrogen, N30:0—30 kg N ha^{-1} before sowing, N15:15—15 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling, N:0:30—30 kg N ha^{-1} at the start of the seed filling, N15:45—15 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N30:30—30 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N45:15—45 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling.

The fertilizer combinations affected the elements of the yield structure. Plant height increased significantly relative to the control following application of nitrogen at 30 kg in its entirety before sowing and application of 60 kg irrespective of the means of application. The highest plants were obtained from the combination with application of 45 kg N before sowing followed by foliar application of 15 kg. Plant height was also increased by sulphur application, but these were minor differences amounting to 4 cm (Figure 4). The interaction of the factors was not shown to significantly influence the height of the soybean plants.

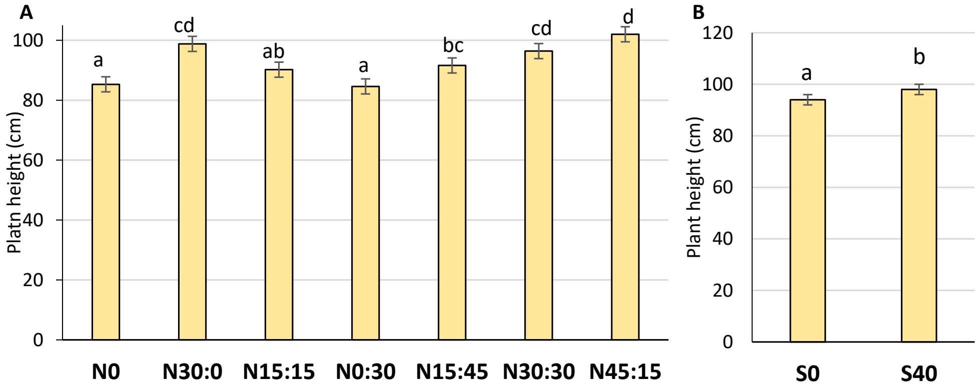

Figure 4. Effects of nitrogen (**A**), sulphur (**B**) application on the height of soybean plants. Means marked with the same letter do not differ significantly at $p = 0.05$, bars mean SE. S0—without sulfur, S40—40 kg S ha^{-1}, N0—without nitrogen, N30:0—30 kg N ha^{-1} before sowing, N15:15—15 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling, N:0:30—30 kg N ha^{-1} at the start of the seed filling, N15:45—15 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N30:30—30 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N45:15—45 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling.

All of the nitrogen fertilizer combinations significantly increased the number of pods per plant in comparison to the control. Sulphur application also increased the pod number per plant, especially in the treatment without nitrogen fertilizer, and in the combination with 30 kg applied 1/2 before sowing +1/2 after emergence or applied in its entirety after emergence (Figure 5). Nitrogen application significantly increased the seed number per pod, except in the case of 30 kg applied 1/2 before sowing +1/2 after emergence. The differences between the other nitrogen combinations were not significant (Figure 6). Sulphur application was not shown to significantly influence the seed number per pod. There was also no significant interaction between application of nitrogen and sulphur.

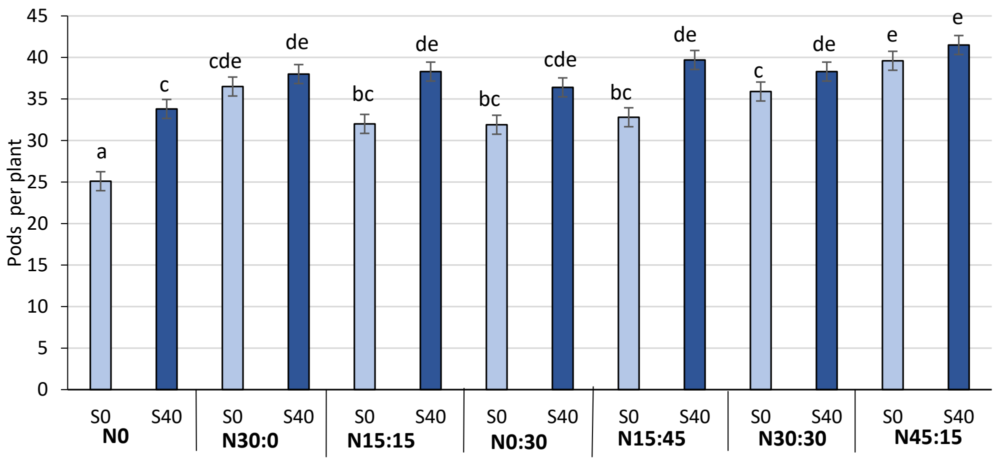

Figure 5. Effects of nitrogen × sulphur application on the number of pods per plant. Means marked with the same letter do not differ significantly at $p = 0.05$, bars mean SE. S0—without sulfur, S40–40 kg S ha^{-1}, N0—without nitrogen, N30:0—30 kg N ha^{-1} before sowing, N15:15—15 kg N ha^{-1}

before sowing + 15 kg N ha^{-1} at the start of the seed filling, N:0:30—30 kg N ha^{-1} at the start of the seed filling, N15:45—15 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N30:30—30 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N45:15—45 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling.

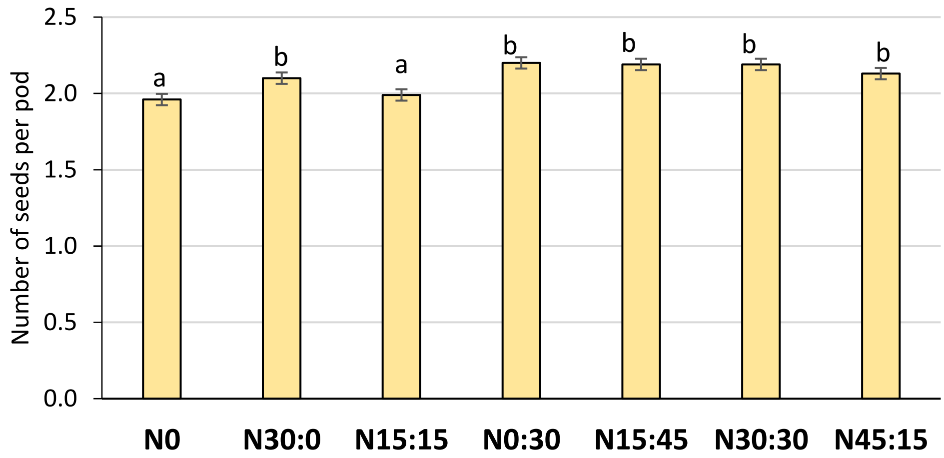

Figure 6. Effects of nitrogen application on the seed number per pod. Means marked with the same letter do not differ significantly at $p = 0.05$, bars mean SE N0—without nitrogen, N30:0—30 kg N ha^{-1} before sowing, N15:15—15 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling, N:0:30—30 kg N ha^{-1} at the start of the seed filling, N15:45—15 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N30:30—30 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N45:15—45 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling.

The nitrogen fertilizer used in the experiment increased the thousand seed weight as well, except in the case of 30 kg applied 1/2 before sowing +1/2 after emergence. The highest value was obtained following application of 60 kg with 1/4 applied before sowing +3/4 after emergence or 1/2 before sowing +1/2 after emergence (Figure 7). Seed yield was positively correlated with the number of pods per plant and the seed number per pod (Table 3).

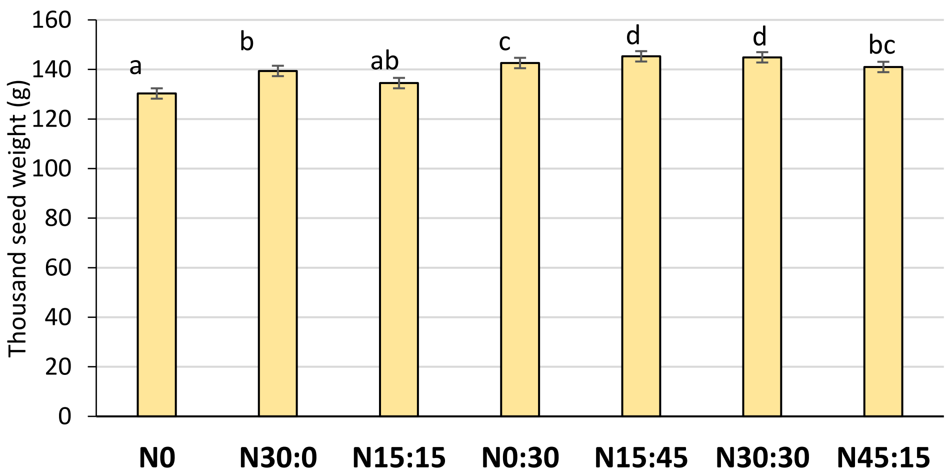

Figure 7. Effects of nitrogen application on the thousand seed weight. Means marked with the same letter do not differ significantly at $p = 0.05$, bars mean SE. N0—without nitrogen, N30:0—30 kg N ha^{-1} before sowing, N15:15–15 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling, N:0:30–30 kg N ha^{-1} at the start of the seed filling, N15:45—15 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N30:30—30 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N45:15—45 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling.

Table 3. Pearson's correlation coefficients between seed yield and selected elements of the yield structure.

Features	Number of Pods per Plant	Seed Number per Pod	Thousand Seed Weight
Yield	0.437 *	0.409 *	0.188

* Significant at $p = 0.05$.

3.3. Content and Yield of Crude Protein and Crude Fat

Nitrogen application increased the crude protein content in the soybean seeds relative to the control, except for 30 kg applied 1/2 before sowing +1/2 after emergence or applied in its entirety after emergence. The differences between the remaining variants of nitrogen application were not significant. Sulphur application increased the content of crude protein only in the treatment without nitrogen and the treatments with lower application of nitrogen, either entirely foliar or 1/2 before sowing +1/2 after emergence. All fertilizer combinations significantly increased the yield of crude protein per hectare relative to the control (Figure 8).

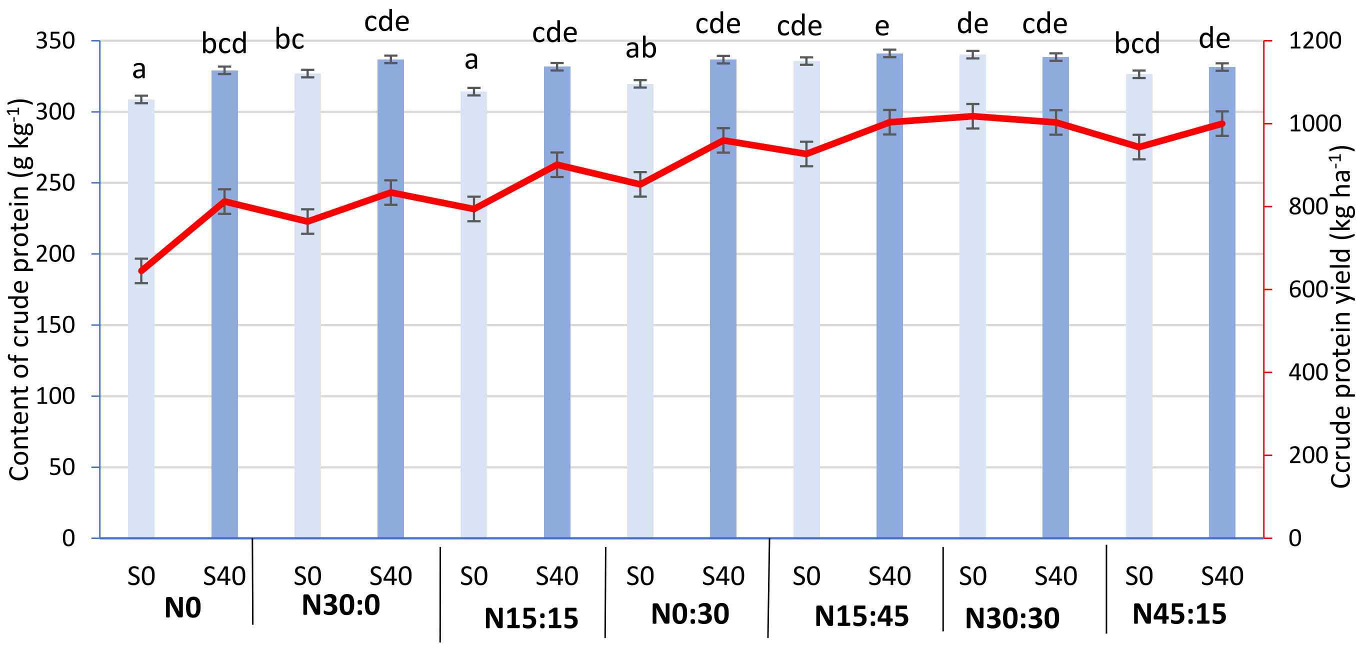

Figure 8. Effects of nitrogen × sulphur application on the content of crude protein in soybean seeds and protein yield. Means marked with the same letter do not differ significantly at $p = 0.05$, bars mean SE, red line–protein yield. S0–without sulfur, S40—40 kg S ha^{-1}, N0–without nitrogen, N30:0—30 kg N ha^{-1} before sowing, N15:15—15 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling, N:0:30—30 kg N ha^{-1} at the start of the seed filling, N15:45—15 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N30:30—30 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N45:15—45 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling.

The lowest crude fat content, 200 g kg^{-1}, was obtained in the soybean seeds from the control treatment. Application of nitrogen and sulphur increased the crude fat content in the soybean seeds. The most beneficial effect on crude fat content in the seeds was obtained in the case of higher nitrogen application (60 kg ha^{-1}), either 1/2 before sowing +1/2 after emergence or 3/4 before sowing +1/4 after emergence, in combination with sulphur application (Figure 9).

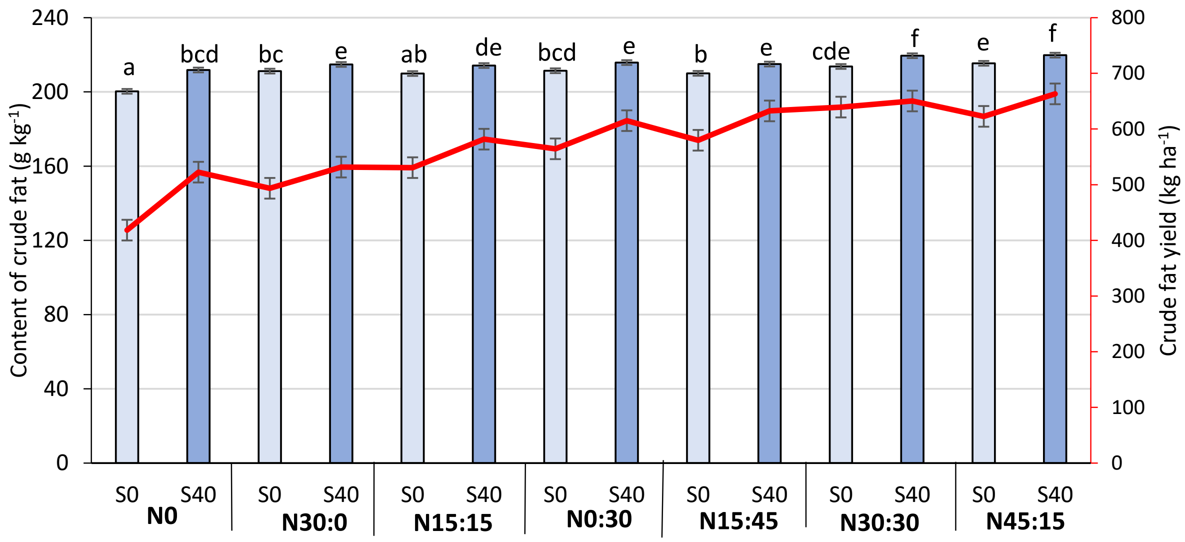

Figure 9. Effects of nitrogen × sulphur application on the content of crude fat in soybean seeds and fat yield. Means marked with the same letter do not differ significantly at p = 0.05, bars mean SE, red line–protein yield. S0—without sulfur, S40—40 kg S ha^{-1}, N0–without nitrogen, N30:0—30 kg N ha^{-1} before sowing, N15:15—15 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling, N:0:30—30 kg N ha^{-1} at the start of the seed filling, N15:45—15 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N30:30–30 kg N ha^{-1} before sowing + 30 kg N ha^{-1} at the start of the seed filling, N45:15—45 kg N ha^{-1} before sowing + 15 kg N ha^{-1} at the start of the seed filling.

3.4. Content of Macroelements

Nitrogen application generally reduced the phosphorus content in the soybean seeds relative to the control, except for 30 kg applied ½ before sowing +½ after emergence. Potassium content in the soybean seeds ranged from 12.40 to 15.42 g kg^{-1} and was highest in the control treatment. Top dressing application of nitrogen in the amount of 30 kg as well as 60 kg applied ½ before sowing + ½ after emergence significantly reduced the content of potassium, and sulphur application in these combinations had no significant effect. Sulphur application reduced potassium content in the soybean seeds when applied in the following nitrogen combinations: N30 (15:15), N60 (15:45) and N60 (45:15)—Table 4.

Table 4. Effect of nitrogen × sulphur application on the content of macroelements in soybean seeds.

Fertilization		Macroelements (g kg^{-1})				
Nitrogen	Sulphur	P	K	Ca	Mg	S
0	0	8.65 e	15.42 i	1.10 a	2.70 a	2.57 a
	40	8.96 f	14.70 g	1.51 d	2.83 a	3.06 c
N30 (30:0)	0	7.96 b	13.68 ef	1.98 g	3.94 fg	2.67 ab
	40	8.12 bc	12.80 c	2.17 h	3.79 de	3.19 cd
N30 (15:15)	0	8.43 de	14.81 gh	1.38 c	3.00 c	2.55 a
	40	8.53 de	12.40 b	1.95 g	2.93 bc	3.31 de
N30 (0:30)	0	7.96 b	14.73 g	1.14 a	3.05 c	2.67 ab
	40	7.87 b	13.90 f	1.70 e	2.98 bc	3.25 cd
N60 (15:45)	0	7.94 b	15.10 hi	1.42 c	3.92 efg	2.70 ab
	40	8.44 de	13.41 de	1.83 f	3.79 def	3.48 e
N60 (30:30)	0	7.58 a	12.44 b	1.86 f	4.04 g	2.64 ab
	40	8.01 b	11.94 a	1.87 f	3.79 def	3.79 f
N60 (45:15)	0	8.38 cd	14.99 gh	1.27 b	3.95 g	2.80 b
	40	8.44 de	13.10 cd	1.55 d	3.71 d	3.93 f

Means in columns marked with the same letter do not differ significantly at p = 0.05.

Nitrogen in the amount of 30 kg applied entirely after emergence or in the amount of 60 kg applied 1/2 before sowing +1/2 after emergence or 3/4 before sowing +1/4 after emergence increased the calcium content in the soybean seeds. In these combinations, sulphur application had no significant effect. In the remaining combinations of nitrogen fertilizer and in the control without nitrogen, sulphur application significantly increased the calcium content in the soybean seeds by 28–47%.

Nitrogen application in the amount of 30 kg, either in its entirety before sowing or 1/2 before sowing +1/2 after emergence, significantly increased the content of magnesium in the soybean seeds by 9.8–13.0% compared to the control. The remaining variants of nitrogen fertilization, N30 (0:30) and N60 irrespective of the means of application, significantly increased the magnesium content in the soybean seeds relative to the control, by 31.0–41.3%, as well as in comparison with 30 kg applied before sowing or in two equal portions, before sowing and after emergence. Sulphur application did not significantly influence magnesium content in the soybean seeds, except in the case of 60 kg N applied 1/2 before sowing +1/2 after emergence or 3/4 before sowing +1/4 after emergence. In these cases, sulphur application decreased magnesium content by 6.0–6.4%.

Sulphur content in the soybean seeds ranged from 2.55 to 3.93 g kg^{-1}. Nitrogen fertilization did not significantly increase sulphur content, except for 60 kg applied 3/4 before sowing +1/4 after emergence. Sulphur application significantly increased the content of this element in the soybeans, both in the control and in every combination of nitrogen application. The highest increase in sulphur content following application of this microelement was observed for the combinations with 60 kg N applied 1/2 before sowing +1/2 after emergence and 3/4 before sowing +1/4 after emergence—by 1.55 g kg^{-1} and 1.13 g kg^{-1}, respectively, i.e., by 43.6% and 40.4%.

4. Discussion

Soybean meets its demand for nitrogen through biological fixation of atmospheric nitrogen as well as from mineral fertilizers. According to [7–9,12] starter nitrogen fertilizer has been shown to increase soybean seed yield. This is confirmed in the present study as well. Nitrogen application before sowing significantly increased seed yield compared to the unfertilized control. The increase in seed yield following application of nitrogen before sowing can be ascribed to the increase in the activity of the root system, rate of photosynthesis, and leaf area index [31,41,42]. Our results are in agreement with the work of Osborne and Riedell [30], who pointed out that soybean grain yield at 16 kg N ha^{-1} starter nitrogen rate, was significantly higher (by 6%) than that at no N treatment with no difference in grain N or oil concentration.

Soybean has a relatively high demand for N, especially during the seed-filling stage. Supply of N to soybean plants during peak demand for seed filling can supplement existing N resources, thereby preventing premature ageing of plants and increasing seed yield [32]. This is confirmed by the present study. The highest seed yield was obtained in the combinations in which 60 kg N ha^{-1} was applied 1/2 before sowing +1/2 after emergence and 3/4 before sowing +1/4 after emergence. In these combinations the seed yield was 8–10 dt ha^{-1} higher than in the combinations without nitrogen application and 5.5–6.5 dt ha^{-1} higher than following application of 30 kg it its entirety before sowing or in two portions, before sowing followed by foliar application. These results are in agreement with research by Zainab et al. [43], who obtained the highest seed yield by applying 60 kg N ha^{-1} in two portions—one quarter before sowing, and the rest at the start of seed-filling. According to Schweiger et al. [44], only 40% to 52% of total nitrogen uptake by soybean originates in symbiotic nitrogen fixation, while the rest is nitrates taken up from the soil. Therefore if total N supply does not meet the needs of soybean, the plants remobilize N accumulated in the leaves to the seeds. This reduces photosynthesis and thus limits the yield potential of soybean [4]. But it is not always the rules. A study conducted in Iowa showed that nitrogen fertilizer applied early reproductive stage had no positive effect on plant DM, grain N concentration and removal, grain yield, or grain quality components at the time of planting had no significant effect on soybean leaf area or grain yield [29].

The increase in seed yield under the influence of nitrogen application was the effect of changes observed in elements of the yield structure, i.e., the pod number per plant and seed number per pod. This was confirmed by the correlation coefficients. Faligowska and Szukała [45] also report that fertilization of soybean with nitrogen has a beneficial effect on elements of the yield structure such as plant height, pod number per plant, and seed weight per plant.

Sulphur performs an important function in the synthesis of protein and essential sulphur-containing amino acids (methionine and cysteine) as well as vitamins and chlorophyll. It is needed for activation of certain enzymes and is an essential component of ferredoxin, which is involved in photosynthesis. Without adequate supply of sulphur, the plants cannot achieve their full yield potential or efficiently utilize applied N [33]. In the present study, application of 40 kg ha^{-1} of sulphur significantly increased the seed yield in the treatment without nitrogen application, by 3.78 dt ha^{-1} (18%), and the pod number per plant by 34.7%. The results are in agreement with those reported by Varun et al. [46] and Burkitbayev et al. [47], who found that seed yield, plant height, number of branches, pod number per plant, and 1000 seed weight of soybean were significantly higher following sulphur application. Similar results were obtained by Dhaker et al. [34], who reported that application of 40 kg S ha^{-1} significantly increased the number of pods per plant, pod length, 1000 seed weight, and seed yield. According to the authors, this may be explained by the fact that sulphur application modifies the physicochemical properties of the soil, thereby improving the availability of nutrients and promoting the growth and development of the plants. This in turn increases translocation of nutrients towards the generative organs and positively affects photosynthesis, and in consequence can substantially increase yields and elements of the yield structure.

Sulphur plays an important role in nitrogen metabolism in the plant, and its deficiency leads to a decrease in nitrogen utilization from fertilizers. According to Jamal et al. [35], S and N interact at the metabolic level in such a way that an imbalance in their availability reduces yields. Therefore S must be included in fertilizer recommendations in soil with S deficiency, and S and N should be administered in balanced quantities in order to obtain optimum yield. In the present study, sulphur application in the treatments in which nitrogen was applied in the amount of 30 kg increased seed yield. In contrast, in the treatments with higher nitrogen application of 60 kg ha^{-1}, sulphur application had no significant effect on seed yield or on most of the elements of the yield structure. This indirectly confirms that the yield-promoting effect of sulphur is mainly due to its effect on nitrogen metabolism. Sulphur performs a special function in nitrogen metabolism, increasing the rate of transformation of nitrogen taken up by the plant into protein. Nitrogen is the nutrient with the greatest yield-promoting effect, and therefore sulphur directly influences seed yield by influencing nitrogen metabolism [48]. In conditions of deficiency of one of these nutrients, the response to fertilization is poor; maximum yield can be achieved when there is an adequate amount of both elements [49]. Proper supply of plants with sulfur is important not only in terms of production, but also ecological. In conditions of sulfur deficiency, fertilizer nitrogen does not perform optimally. The effective use of nitrogen by plants is also important for the environment, because in conditions of sulfur deficiency, nitrogen may be lost as a result of nitrates penetrating into groundwater, as well as volatilization of gaseous forms into the atmosphere [50].

Soybean seeds have high content of protein and fat. The protein content in legume plants is influenced by genetic factors, i.e., the properties of the cultivar, but also by agricultural procedures, particularly the use of nitrogen fertilizer [4,9,11,12]. This was confirmed in the present study. Application of 60 kg of nitrogen significantly increased the protein content in the soybean seeds relative to the unfertilized control treatment. However, the effect of 30 kg of nitrogen was significant only when the full amount was applied before sowing. Both nitrogen and sulphur fertilization increased oil content in the seeds. Sulphur application also had a beneficial effect by increasing protein content in the seeds, but only in conditions without nitrogen fertilizer or the lower level of nitrogen applied in its entirety to the leaves or 1/2 before sowing + 1/2 after emergence. The beneficial effect of nitrogen application on protein in soybean seeds is confirmed by other authors [8,11]. However, Fecak et al. [51] found no significant changes in the content of total protein in soybean seeds after the application of differentiated nitrogen fertilization.

According to Kozłowska-Strawska and Kaczor [49], the main consequence of sulphur deficiency is reduced protein synthesis. Sulphur is an essential nutrient for the activity of enzymes taking part in nitrate reduction [52]. Therefore, plants grown in conditions without this element accumulate nitrogen in non-protein form (nitrates, amides and other compounds). Plants of the *Fabaceae* family, which are a valuable source of protein for people and animals, require adequate fertilization with sulphur [25]. Without this nutrient, they produce protein with much lower content of sulphur-containing amino acids, especially methionine, which is one of the most valuable amino acids determining the nutritional value of plants [22,36]. Sulphur is also involved in fat synthesis, and due to its metabolic functions it has an important influence on the quantity and quality of oil accumulated in the seeds. Sulphur compounds, such as vitamin H with coenzyme A, form an enzymatic system essential to synthesis of fatty acids. The results of research by other authors confirm that sulphur application increases the content of protein and fat in soybean seeds [35,37].

Soybean is an important source not only of protein and fats, but also of minerals such as potassium, magnesium, calcium, phosphorus, and iron [3,4,53]. In the present study, nitrogen application generally did not affect the content of sulphur and calcium in the soybean seeds, but it reduced the content of phosphorus and potassium (only in the case of foliar application of 30 kg and 60 kg (30:30) while increasing the content of magnesium, especially the higher rate of application. Sulphur application did not affect the content of phosphorus in the seeds but increased that of calcium and sulphur and decreased that of magnesium, although only in the combinations with higher nitrogen application. The findings of other authors are varied. According to Jarecki and Bobrecka-Jamro [4] and Shorabi et al. [32], nitrogen application did not significantly affect the content of phosphorus or potassium in soybean seeds. An increase in calcium content in the grain of cereals following sulphur application was reported by Brodowska and Kaczor [54]. Barczak et al. [14] observed a decrease in phosphorus content in response to application of various amounts of sulphur in an experiment on lupin. However, according to the authors, this may have been caused by 'dilution' of phosphorus as a result of the increased yield resulting from sulphur application, and not by the antagonistic interaction of phosphate ion (V) and sulphate ion (VI). A similar explanation of decreased content of minerals is given by Cakmak [55].

5. Conclusions

Application of nitrogen and sulphur significantly increased the seed yield of soybean. The most beneficial effect was obtained in the combinations with 60 kg N applied 1/2 before sowing +1/2 after emergence and 3/4 before sowing +1/2 after emergence. In these combinations, sulphur application did not significantly affect seed yield. In the remaining combinations of nitrogen application, sulphur application significantly increased the seed yield. Taking into account the yield and the chemical composition of the soybean seeds, fertilization with 60 kg N ha^{-1} in two portions can be recommended—1/2 or 3/4 before sowing and the remainder during the development of pods and seeds—in combination with sulphur application. In further research, it is worth paying attention to the form of sulfur introduced in fertilizers. An interesting issue is also the effect of sulfur fertilization on the composition of fatty acids and the biological value of protein.

Author Contributions: Conceptualization, A.G.; methodology, A.G., A.K.-D. and E.J.; investigation, A.G., E.J. and A.K.-D.; writing—original draft preparation, A.G., E.J., E.F.-O. and A.K.-D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Minister of Education and Science of Poland, grant number RKS/S/55/22.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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