agronomy

MDPI

Article

Nitrogen Uptake from Different Sources by Non-GMO Soybean Varieties

Katarzyna Rymuza *, Elżbieta Radzka and Andrzej Wysokiński

Faculty of Agrobioengineering and Animal Husbandry, Siedlce University of Natural Sciences and Humanities, ul. Prusa 14, 08-110 Siedlce, Poland; elzbieta.radzka@uph.edu.pl (E.R.); andrzej.wysokinski@uph.edu.pl (A.W.)

* Correspondence: katarzyna.rymuza@uph.edu.pl; Tel.: +48(25)6431246

Received: 30 July 2020; Accepted: 17 August 2020; Published: 19 August 2020

Abstract: Soybean has the ability to live in symbiosis with microorganisms and take up nitrogen from the atmosphere, fertiliser and soil reserves. The amount of nitrogen taken up from these sources depends on many biotic and abiotic factors, e.g., the rhizobium species, cultivar, as well as weather and agricultural conditions. A field experiment was conducted in eastern Poland (central Europe) in two successive growing seasons to examine the uptake of nitrogen from the atmosphere (NDFA—% nitrogen derived from the atmosphere), fertiliser (NDFF—% of nitrogen derived from fertiliser) and soil reserves (NDFS—% of nitrogen derived from the soil) for three non-GMO (non genetically modified organism) soybean cultivars: Abelina, SG Anser and Merlin. Pre-plant fertilisation of plants with nitrogen excess with the ^{15}N isotope and the isotope dilution method were applied. Soil reserves and the atmosphere were major nitrogen sources for soybean. Soybean roots contained the most atmosphere-derived nitrogen (45.85%), the amount being lower for soil reserves (41.43%) and the lowest for fertiliser (12.72%). Harvest residues and seeds contained the most soil reserve-derived nitrogen, the amount being lower for the atmospheric nitrogen and the lowest for fertiliser-derived nitrogen. The amount of nitrogen derived from different sources in the whole soybean mass significantly depended on cultivars and years' percentage values being affected by study years only. Less atmospheric nitrogen was accumulated in cv. Abelina roots (2.15 kg N·ha^{-1}) compared with cv. SG Anser (3.07 kg N·ha^{-1}) or cv. Merlin (2.89 kg N·ha^{-1}). More atmospheric nitrogen was recorded in the post-harvest residues and seeds of cv. Abelina and SG Anser than Merlin. The content of soil reserve-derived nitrogen taken up by the whole soybean plants averaged 61.29 kg N·kg^{-1}, the amounts being 50.95 and 11.38 kgN·kg^{-1} for nitrogen taken up from the atmosphere and fertiliser, respectively. Soybean grown in the study year with more favourable thermal and precipitation conditions (2017) took up more nitrogen from all the sources compared with the year 2018.

Keywords: nitrogen fixation; *Glycine max* (L.) *Merr.*; fertiliser; isotope ^{15}N; legume

1. Introduction

Modern intensive agriculture heavily relies on nitrogen fertilisers in order to achieve high production and economic effects. Their application generates both economic and environmental costs, the latter being mainly due to pollution of watercourses and bodies of water with soluble forms of nitrogen compounds, as well as emission to the atmosphere of excessive amounts of nitrogen oxides [1]. Reduced excessive mineral fertilisation generates lower demand, which reduces fertiliser production and transportation, thus reducing greenhouse gas emissions. In this way, sustainable development objectives are met. As far as agricultural production is concerned, this aim is achieved by applying fertilisers and processes of natural origin, with the biological nitrogen fixation process (BNF) being one of such processes. The process allows for an introduction into the soil environment of the most important yield-forming element, that is nitrogen [2,3]. The nitrogen fixation process is also important due to economic reasons. Nitrogen-fixing plants increase the soil availability of this element and, thus, contribute to an increase in the yield of the crops that follow, while simultaneously reducing outlays associated with mineral fertilisers [3,4]. Chemically, the biological nitrogen fixation process is a conversion of a plant- and animal-unavailable form of elemental nitrogen N2 to the reduced form—ammonia—which may be further metabolised in the cells of living organisms [5–8]. In nature, the nitrogen fixation process involves participation of microorganisms which differ in morphological and physiological terms, their habitat requirements and complexity of the system in which the N2 assimilation process is carried out [5,6,9,10]. Some microorganisms are able to independently fix nitrogen in the soil environment while others fix this element in symbiosis. The most important crop plants which live in symbiosis with bacteria fixing free atmospheric nitrogen include leguminous plants, with soybean being the most outstanding example [11–14]. The residues of these crop plants remaining in the field improve soil structure and affect its fertility [15–17] and are a nitrogen source for the following crop [18–20]. Thus, it is important to determine the amount of nitrogen which was assimilated and taken up by various plant parts. The amount of fixed nitrogen is also cultivar-related [21,22].

An increase in non-GMO soybean demand in the European Union (EU) has been observed. More and more countries are considering cultivation of non-GMO cultivars on a larger scale. Cultivated cultivars should be certified as free of any genetic modifications [23,24].

Research on nitrogen uptake by soybean was mainly conducted in countries with the highest soybean production, such as Brazil, Argentina and North America [6,7,22,25,26]. Such research is scarce in Europe [27,28]. As possibilities of soybean cultivation under European conditions are increasing, due to climate change and changing consumer awareness, research into this crop plant seems to be reasonable from the practical point of view as well. Availability of information on improved soybean cultivars and their agrotechnology as well as benefits associated with its cultivation will allow farmers to meet the market demand and expectations of producers.

The objective of the research reported here was to determine the amount of nitrogen taken up from the atmosphere, mineral fertiliser and soil reserves by three new soybean cultivars cultivated in central Europe. It was assumed that the atmosphere would be the major nitrogen source for soybean. Moreover, it was examined as to what quantity of nitrogen obtained from individual sources was removed from the field with seed yield and was introduced into the soil with the post-harvest residues of the test cultivars.

2. Materials and Methods

2.1. Description of the Experiment

A field experiment was conducted in Łączka, eastern Poland (N52°15′, E21°95′), in 2017–2018. The experimental design was randomized blocks with three replicates. The following three non-GMO second early (OOO++) soybean cultivars were examined: Abelina, SG Anser and Merlin. Cultivar selection was affected by the region's climatic conditions, so soybean was to be harvested no later than mid-October. The producer and only supplier of these cultivars is Saatbau Polska Sp. z o.o., which holds a certificate confirming that every batch of the produced and offered planting material is free of GMO pollutants. The planting material is treated with nodulating bacteria using the Fix Fertig technology, rendering it ready for planting. In Fix Fertig technology during the technological process, seeds are coated with the nodulating bacteria (*Bradyrhizobium japonicum*, NPPL HiStick) and glue, which is also a preserving and protecting agent. Nodulation ensures an accurate distribution of the rhizobia over the soybean seeds and guarantees nodule development. Pre-inoculated soybeans score highly in terms of nitrogen fixation in the nodules, ensuring the best results for following crops in crop rotation. The soil of the experimental site was classified as representing the Haplic Luvisol group according to the World Reference Base for Soil Resources (2014) [29]. Selected properties of the soil are shown in Table 1. The soil had average organic carbon and total nitrogen contents as well as average phosphorus content, high potassium content and low magnesium content, all in terms of plant available forms.

Table 1. Some soil properties in the layer 0–0.25 m prior to the experiment set-up in 2017 and 2018.

Soil Properties	Unit	Year	
		2017	2018
pH (in KCl)	-	6.9	7.1
C_{org}	$g \cdot kg^{-1}$	9.0	8.9
N_t		0.8	0.8
Fe_t	$mg \cdot kg^{-1}$	995	990
B_t		0.7	0.7
P_{av}		55.8	57.1
K_{av}		132.8	130.3
Mg_{av}		26.5	25.9

The isotope dilution method was used in the study: the method requires the use of mineral fertilisers enriched with the isotope ^{15}N and a simultaneous cultivation of a control plant unable to live in symbiosis with nodulating bacteria. Soybean was grown in microplots whose area was 1 m^2 and which were established in fields planted to respective cultivars. Maize (*Zea mays* L.), grown in plots whose area was 4 m^2 (2×2 m) and under the same conditions (in the same field and in three replicates), was the control plant. Both the crop plants were fertilised with nitrogen applied as ammonium sulphate $(NH_4)_2SO_4$ enriched with 5 atom% isotope ^{15}N and at the rate introduced into the soil of 30 kg $N \cdot ha^{-1}$ (3 g $N \cdot m^{-2}$). P and K rates were set based on the soil content of plant-available forms of these elements (Table 1) and they amounted to 30 kg P and 90 kg K per 1 ha. Each year, soybean was preceded by maize. The crop was grown in a conventional soil cultivation system.

Both soybean and maize were sown manually in early May (on the 4th and 5th of May) at the between-row spacing of 22 cm and the depth of 4 cm, the amount of seeds being 70 and 75 (for soybean and maize, respectively) per 1 m^2. No herbicides were applied to control weeds, which were removed by hand. The whole test plants (both soybean and maize) were harvested by hand in late September at the stage of full maturity (BBCH 99) by digging them out with a spade to the depth of 0.25 m. Plant parts were separated into roots, above-ground post-harvest residues and seeds. The above-ground residues consisted of all the above-ground soybean and maize parts, excluding seeds. After removal of seeds, the pods were included into post-harvest residues. Next, the collected mass was weighed and representative samples of the three groups were taken.

2.2. Chemical Analyses

In all the plant samples, the following parameters were determined: dry matter content (D.M.) by the gravimetric method at 70 °C, total nitrogen content by the Kjeldahl method and enrichment in the ^{15}N isotope by the emission spectrometer NOI-6e. The percentage of nitrogen derived from different sources: atmosphere—NDFA, mineral fertilizer—NDFF and soil—NDFS, in soybean was calculated using the formulas given by Azam and Farooq [30] and Kalembasa et al. [31]:

(a) Percentage of nitrogen derived from the atmosphere:

$$\%NDFA = \left[1 - \frac{at\%^{15}Nenriched.fx}{at\%^{15}Nenriched.nfx}\right] \cdot 100 \quad (1)$$

where:

%NDFA—% of nitrogen derived from the atmosphere,

at% ^{15}*Nenriched. fx*—^{15}N isotope excess in soybean,
at% ^{15}*Nenriched. nfx*—^{15}N isotope excess in the control plant—maize.

(b) Percentage of nitrogen derived from fertiliser:

$$\%NDFF = \left[1 - \frac{at\%^{15}Nenriched.fx}{at\%^{15}Nenriched.fert.}\right] \cdot 100 \quad (2)$$

where:

%NDFF—% of nitrogen derived from fertiliser,
at% ^{15}*Nenriched. fx*—^{15}N isotope excess in soybean,
at% ^{15}*Nenriched. fert.*—^{15}N isotope excess of fertiliser.

(c) Percentage of nitrogen derived from soil:

$$\%NDFS = 100 - (\%NDFA + \%NDFF) \quad (3)$$

where:

%NDFS—% of nitrogen derived from the soil,
%NDFA—% of nitrogen derived from the atmosphere,
%NDFF—% of nitrogen derived from the fertilizer.

The nitrogen pool, conventionally called 'from soil'—NDFS, includes all other sources apart from the atmosphere and mineral fertiliser.

2.3. Statistical Analyses

The results of the experiments were analysed by analysis of variance (ANOVA). Significance of sources of variation was checked with the Fisher–Snedecor test, and mean values were separated with the Tukey's test at the significance level of $p < 0.05$. For these calculations, the Statistica 13.0PL (StatSoft, Tulsa, OK, USA) was used.

2.4. Weather Conditions

Precipitation and thermal conditions during the study period are shown in Figure 1.

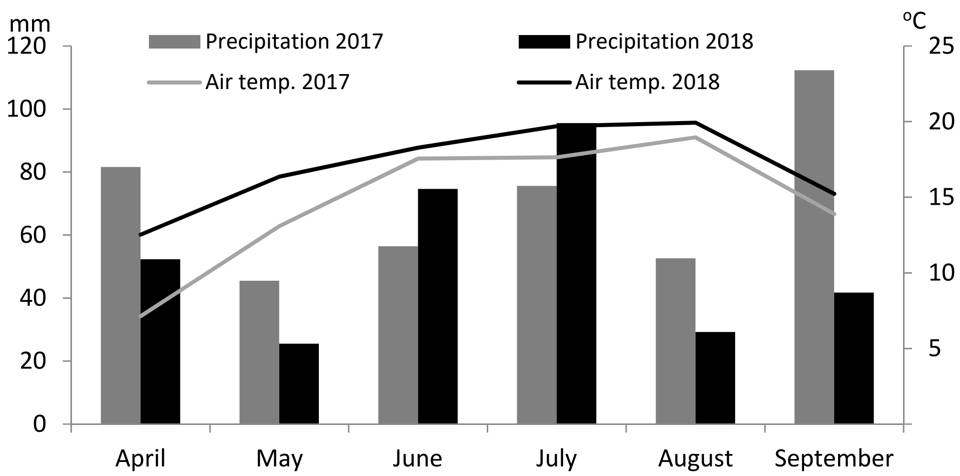

Figure 1. Precipitation and air temperatures (temp.) in 2017–2018 according to IMWM-NRI (Institute of Meteorology and Water Management—National Research Institute) Warsaw.

Analysis of weather patterns in both the soybean and maize growing seasons revealed that the precipitation and thermal conditions in individual study years were changeable (Figure 1). The highest precipitation sums in 2017 and 2018 were recorded in, respectively, September (112 mm) and July (96 mm). By contrast, the lowest precipitation sums in 2017 and 2018 were recorded in May (46 and 26 mm, respectively) and September (53 and 29 mm, respectively). Low precipitation in May 2018 was unconducive to good plant germination and emergence. The growing season in 2018 was much warmer than in 2017. The average monthly air temperature in all the months (April–October) of 2018 was higher compared with 2017. In both the study years, August was the warmest month of the soybean growing season, its average temperature amounting to 19.0 and 19.9 °C.

3. Results

3.1. Yield, Nitrogen Content, Enrichment in ^{15}N and Total Amount of Nitrogen Taken Up by Different Soybean Parts

The amount of dry matter of individual soybean parts was affected by the growing season and cultivar. A significantly higher root, post-harvest residue and seed mass were recorded in 2017 compared with 2018. Across the two study years, the root mass was higher in cv. SG Anser (0.59 Mg·ha^{-1}) compared with Abelina or Merlin, with the means for the two cultivars being similar. In terms of root yield, differences between cultivars were affected by conditions during the growing season, as shown by significance of the interaction of cultivars x years. In 2017, the root yield of cv. Merlin was lower compared with the remaining cultivars, whereas in 2018, cv. Merlin and Abelina produced similar root yields (respectively 0.46 and 0.40 Mg·ha^{-1}), which were significantly lower compared with cv. SG Anser (0.55 Mg·ha^{-1}). Regardless of the study years, seed yield and residue yield produced by cv. Abelina and SG Anser were higher compared with cv. Merlin. The total biomass amounts produced by cv. Abelina and SG Anser were about 40% higher compared with cv. Merlin (Table 2).

Table 2. The amount of soybean dry matter (D.M.), Mg·ha^{-1}.

Plant Part	Cultivar				Mean
	Year	**Abelina**	**SG Anser**	**Merlin**	
roots	2017	0.57 a	0.64 a	0.53 b	0.58 *
	2018	0.40 b	0.55 a	0.46 b	0.47 *
	Mean	0.49 B	0.59 A	0.50 B	0.53
post-harvest residues	2017	4.87	4.48	3.31	4.22 *
	2018	3.03	3.17	1.98	2.72 *
	Mean	3.95 A	3.82 A	2.65 B	3.473
seeds	2017	2.86	2.73	1.78	2.46 *
	2018	2.01	1.96	1.61	1.86 *
	Mean	2.43 A	2.34 A	1.70 B	2.16
total	2017	8.31	7.84	5.63	7.26 *
	2018	5.45	5.67	4.05	5.06 *
	Mean	6.88 A	6.76 A	4.84 B	6.16

A, B—values followed by the same letters indicate cultivars which differ insignificantly at $p < 0.05$. a, b—values followed by the same letters indicate cultivars which differ insignificantly in a given year. Means for years followed by an asterisk (*—in rows) differ significantly at $p \leq 0.05$.

Regardless of cultivar or growing season, the highest nitrogen content was determined in plant seeds (45.5 g·kg^{-1}), the contents in roots and residues being respectively 11.06 and 5.54 g·kg^{-1}. The soybean seed content of nitrogen was influenced by weather conditions during the study years only. More nitrogen was accumulated by cultivars in all the plant parts in 2017 than 2018. No interaction between study years and cultivars was confirmed, which means that nitrogen accumulation by the experimental cultivars in their roots, post-harvest residues and seeds under certain weather conditions was similar. A higher nitrogen content was found in roots, post-harvest residues and the total biomass of cv. Merlin compared with cv. Abelina or SG Anser (Table 3).

Table 3. Nitrogen (N) content in different soybean parts, g·kg^{-1}.

Plant Part	Cultivar				Mean
	Year	Abelina	SG Anser	Merlin	
roots	2017	10.40	11.82	13.38	11.87 *
	2018	10.54	9.23	11.00	10.26 *
	Mean	10.47 B	10.53 B	12.19 A	11.06
post-harvest residues	2017	5.51	5.34	6.70	5.85 *
	2018	4.83	5.10	5.73	5.22 *
	Mean	5.17 B	5.22 B	6.22 A	5.54
seeds	2017	45.63	48.58	48.97	47.73 *
	2018	42.57	44.27	42.97	43.27 *
	Mean	44.10	46.43	45.97	45.5
total	2017	19.68	20.91	20.78	20.46 *
	2018	10.09	10.26	12.13	10.83 *
	Mean	14.89 B	15.59 A,B	16.46 A	15.64

A, B—values followed by the same letters indicate cultivars which differ insignificantly at $p < 0.05$. a, b—values followed by the same letters indicate cultivars which differ insignificantly in a given year. Means for years followed by an asterisk (*—in rows) differ significantly at $p \leq 0.05$.

Regardless of cultivar or growing season, the highest mean enrichment in the isotope ^{15}N was found in roots (1.27%), the mean values for post-harvest residues and seeds amounting to 1.22 and 0.83%, respectively. Enrichment in the isotope ^{15}N for individual soybean parts and the total biomass was higher in 2017 than 2018. There were no significant differences between the cultivars in this respect. Mean enrichment in the total biomass of the cultivars ranged from 0.89% in cv. Abelina to 0.91% in cv. Merlin. Seed enrichment of the test cultivars was affected by the growing conditions. In 2017, seeds of all the cultivars took up similar amounts of ^{15}N whereas in 2018 the enrichment in seeds was lower in cv. Abelina than Merlin (0.76 vs. 0.84%) (Table 4).

Table 4. Soybean enrichment in the isotope ^{15}N, %.

Plant Part	Cultivar				Mean
	Year	Abelina	SG Anser	Merlin	
roots	2017	1.44	1.33	1.29	1.35 *
	2018	1.30	1.09	1.18	1.19 *
	Mean	1.37	1.21	1.24	1.27
post-harvest residues	2017	1.29	1.19	1.26	1.25 *
	2018	1.17	1.15	1.23	1.18 *
	Mean	1.23	1.17	1.25	1.22
seeds	2017	0.86 a	0.91 a	0.84 a	0.87 *
	2018	0.76 b	0.79 a,b	0.84 a	0.80 *
	Mean	0.81	0.85	0.84	0.83
total	2017	0.95	0.97	0.95	0.96 *
	2018	0.84	0.85	0.91	0.87 *
	Mean	0.89	0.91	0.93	0.91

a, b—values followed by the same letters indicate cultivars which differ insignificantly in a given year. Means for years followed by an asterisk (*—in rows) differ significantly at $p \leq 0.05$.

The highest total nitrogen accumulation was determined in soybean seeds (98.61 kg N·ha^{-1}), with it being the lowest in roots (5.82 kg N·ha^{-1}). Regardless of the cultivar, the total amount of nitrogen taken up by roots, post-harvest residues and seeds was higher in 2017 than 2018. Cv. Abelina and SG Anser accumulated more nitrogen in their post-harvest residues and seeds than cv. Merlin (Table 5).

Table 5. Total amount of nitrogen taken up by soybean, kg N·ha^{-1}.

Plant Part	Cultivar				Mean
	Year	Abelina	SG Anser	Merlin	
roots	2017	6.02	7.53	7.10	6.88 *
	2018	4.21	5.05	5.02	4.76 *
	Mean	5.11	6.29	6.06	5.82
post-harvest residues	2017	26.89	23.84	22.31	24.34 *
	2018	14.70	16.19	11.33	14.07 *
	Mean	20.79 A	20.01 A	16.82 B	19.21
seeds	2017	130.63	132.24	87.37	116.74 *
	2018	85.38	86.76	69.27	80.50 *
	Mean	108.00 A	109.50 A	78.32 B	98.61
total	2017	163.54	163.60	116.78	147.97 *
	2018	104.29	107.99	85.61	99.30 *
	Mean	133.91 A	135.80 A	101.20 B	123.63

A, B—values followed by the same letters indicate cultivars which differ insignificantly at $p < 0.05$. a, b—values followed by the same letters indicate cultivars which differ insignificantly in a given year at $p \leq 0.05$. Means for years followed by an asterisk (*—in rows) differ significantly at $p \leq 0.05$.

3.2. Amount of Nitrogen Taken up by Soybean Cultivars from the Atmosphere, Soil Reserves and Mineral Fertiliser

Similar to the characteristics described above, the amount of nitrogen taken up from the atmosphere by soybean plants was higher in 2017 than 2018. Regardless of cultivar or growing season, nitrogen accumulation was the highest in seeds (40.43 kg N·ha^{-1}, on average) and the lowest in roots (2.71 kg N·ha^{-1}). The amount of nitrogen taken up from the atmosphere by different plant parts and the whole biomass was affected by the cultivar. The average atmospheric nitrogen accumulation in the total biomass was higher for cv. Abelina and SG Anser (56.72 and 55.15 kg N·ha^{-1}, respectively) compared with cv. Merlin (40.99 kg N·ha^{-1}). The amount of nitrogen acquired from the atmosphere and stored in the total biomass of the cultivars was also influenced by the study years. In 2017, atmospheric nitrogen uptake was the highest for cv. Abelina (72.82 kg N·ha^{-1}), lower for cv. SG Anser (69.86 kg N·ha^{-1}) and the lowest for cv. Merlin (53.30 kg N·ha^{-1}). In 2018, the quantity of nitrogen taken up from the atmosphere by the total biomass of cv. Abelina and SG Anser was similar (40.62 and 40.44 kg N·ha^{-1}, respectively) and significantly higher compared with cv. Merlin (28.67 kg N·ha^{-1}). The lowest atmospheric nitrogen accumulation was determined in cv. Abelina roots (2.15 kg N·ha^{-1}). Roots of cv. SG Anser and Merlin took up similar quantities of atmospheric nitrogen. The average nitrogen uptake from the atmosphere across the two study years was the lowest in post-harvest residues and seeds of cv. Merlin. The amount of nitrogen accumulated in the seeds of the examined cultivars was affected by the study years, as indicated by a significant interaction between study years and cultivars. In 2017, the lowest atmospheric nitrogen accumulation was recorded for seeds of cv. Merlin, with it being higher for cv. SG Anser and the highest for cv. Abelina. In 2018, cv. Abelina and SG Anser accumulated similar amounts of nitrogen in their seeds. The quantity of nitrogen taken up from fertiliser and soil reserves by the roots and post-harvest residues of the test cultivars was similar. In contrast, between-cultivar differences as to nitrogen amounts acquired from fertiliser and soil were recorded for seeds. When averaged across the two study years, an accumulation in seeds was the lowest for cv. Merlin taking up nitrogen from fertiliser (6.60 kg N·ha^{-1}) and soil (40.31 kg N·ha^{-1}). Nitrogen uptake from fertiliser and soil by seeds of the test cultivars was influenced by study years. In 2017, seeds of cv. Merlin accumulated nearly twice as little nitrogen from fertiliser and soil as the remaining cultivars. In 2018, seeds of cv. Abelina, SG Anser and Merlin took up similar nitrogen quantities from fertiliser and soil (Figures 2–6).

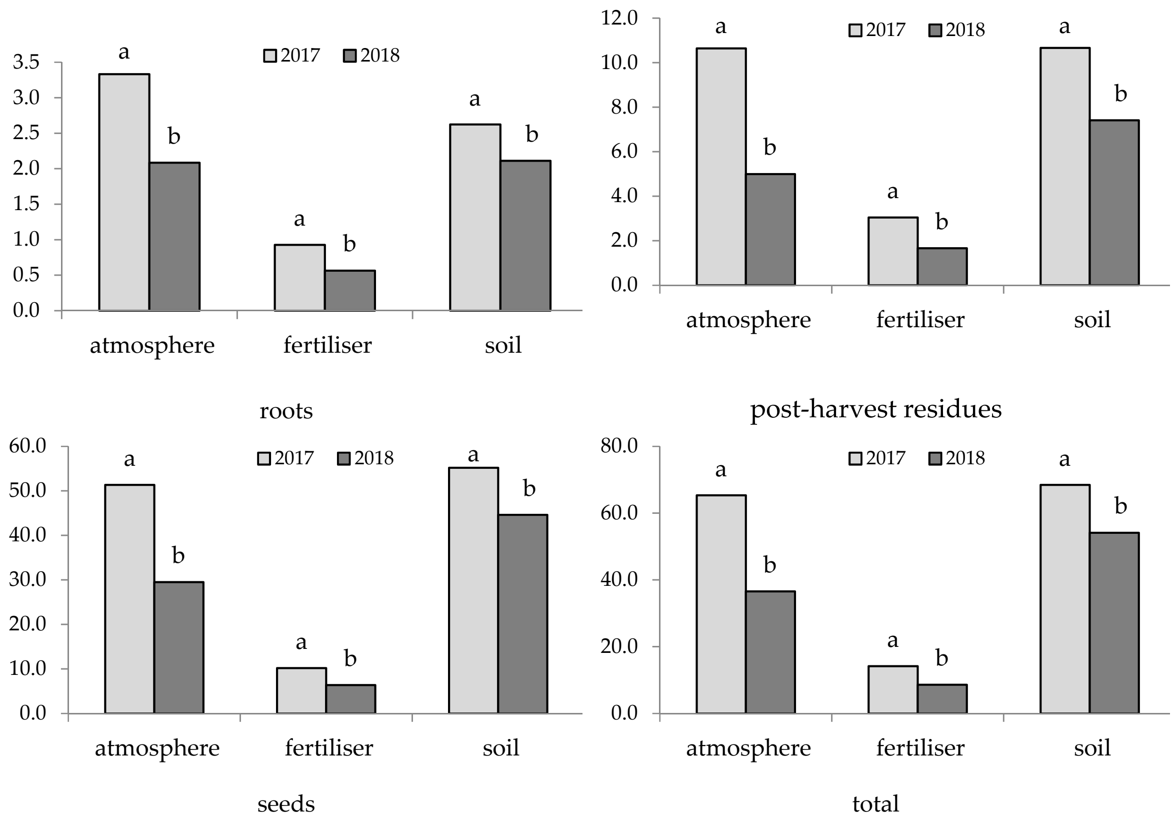

Figure 2. Amount of nitrogen taken up by the plant parts from various sources in the study years, kg N·ha^{-1}. a, b—values followed by the same letters indicate years which differ insignificantly at $p \leq 0.05$.

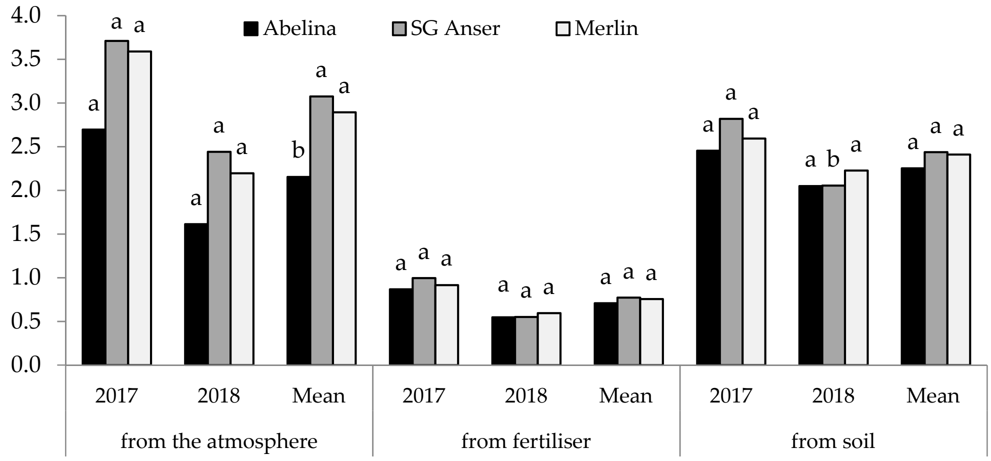

Figure 3. Amount of nitrogen taken up by soybean roots from various sources in the study years, kg N·ha^{-1}. a, b—values followed by the same letters indicate cultivars which differ insignificantly in a given year at $p \leq 0.05$.

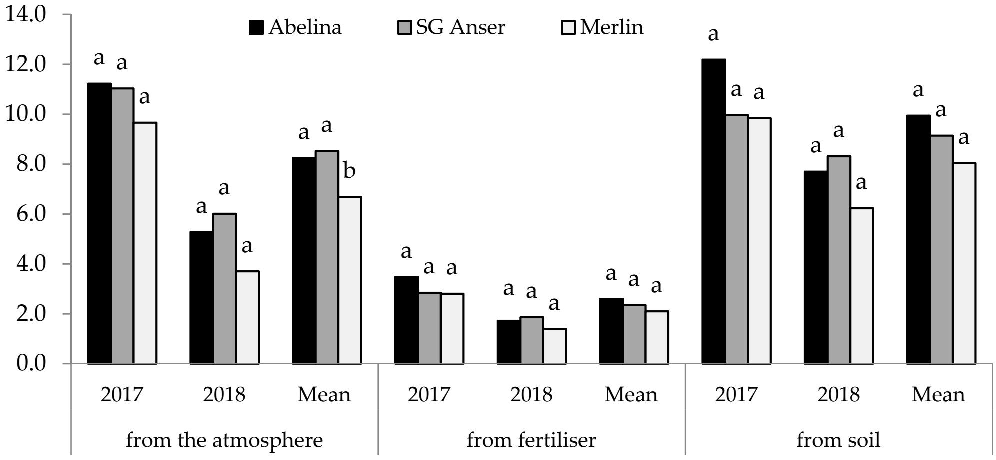

Figure 4. Amount of nitrogen taken up by soybean post-harvest residues from various sources in the study years, kg N·ha^{-1}. a, b—values followed by the same letters indicate cultivars which differ insignificantly in a given year at $p \leq 0.05$.

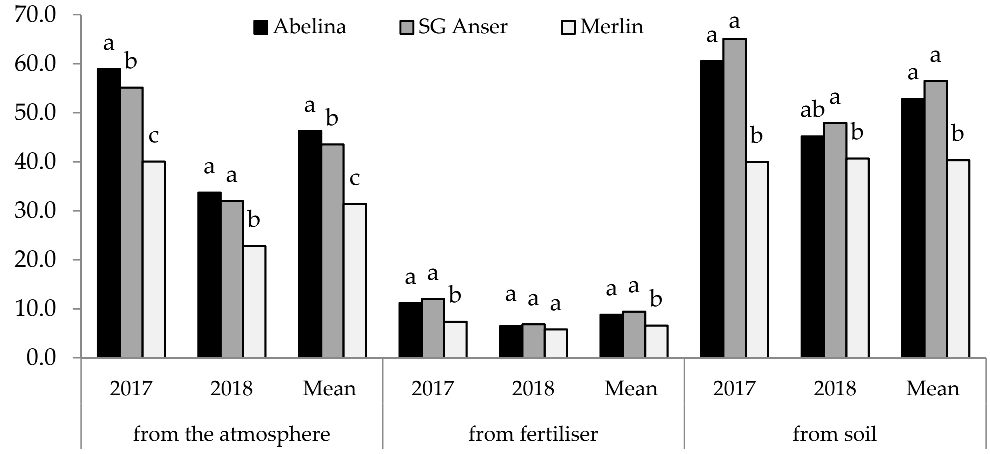

Figure 5. Amount of nitrogen taken up by soybean post-harvest residues from various sources in the study years, kg N·ha^{-1}. a, b, c—values followed by the same letters indicate cultivars which differ insignificantly in a given year at $p \leq 0.05$.

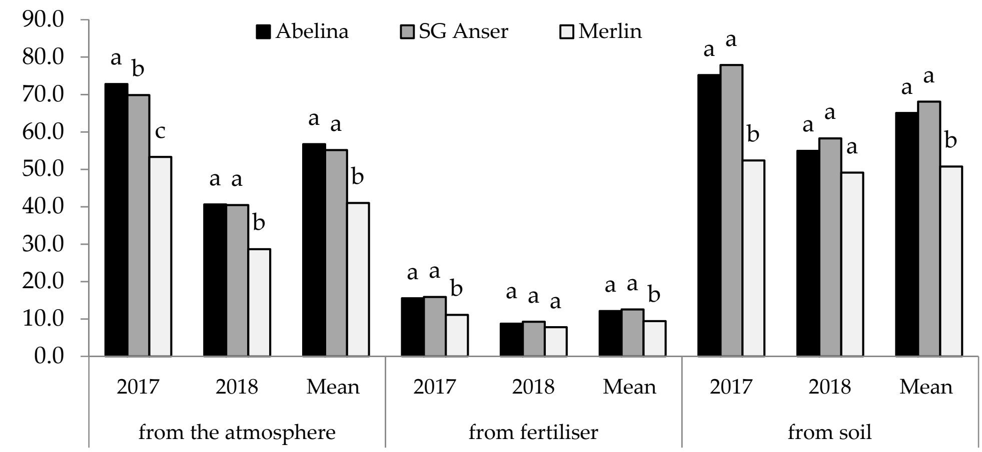

Figure 6. Amount of nitrogen taken up by the whole plants of test soybean cultivars from various sources in the study years, kg N·ha^{-1}. a, b, c—values followed by the same letters indicate cultivars which differ insignificantly in a given year at $p \leq 0.05$.

3.3. Percentage of Nitrogen Taken up by Soybean Cultivars from the Atmosphere, Soil Reserves and Mineral Fertiliser

The percentage of nitrogen taken up from the atmosphere and accumulated in soybean roots, post-harvest residues and seeds was 45.85%, 39.58% and 40.34%, respectively. Moreover, the percentage was higher in 2017 than 2018. A higher percentage of nitrogen taken up from the atmosphere by roots was recorded for cv. SG Anser (48.73%) compared with Abelina (41.56%). As far as post-harvest residues were concerned, the percentage was higher for cv. SG Anser (41.68%) than cv. Merlin (38.06%). The percentage of nitrogen derived from the atmosphere in the seeds and the total biomass of test cultivars was affected by growing conditions. In 2017, the percentage of nitrogen taken up from the atmosphere by seeds and the total biomass of test cultivars was similar. In 2018, the percentage was lower for the biomass of cv. Merlin (33.46%) compared with Abelina (38.95%) and SG Anser (37.57%). In seeds, the percentage of atmospheric nitrogen for cv. Merlin was lower than cv. Abelina (39.52%) and similar to cv. SG Anser (37.00%) (Table 6).

Table 6. Percentages of nitrogen taken up by soybean from the atmosphere, %.

Plant Part	Cultivar				Mean
	Year	Abelina	SG Anser	Merlin	
roots	2017	44.83	49.04	50.58	48.15 *
	2018	38.29	48.41	43.93	43.55 *
	Mean	41.56 B	48.73 A	47.26 A,B	45.85
post-harvest residues	2017	41.89	46.25	43.24	43.79 *
	2018	36.12	37.11	32.87	35.37 *
	Mean	39.01 A,B	41.68 A	38.06 B	39.58
seeds	2017	45.09 a	41.67 a	45.94 a	44.23 *
	2018	39.52 a	37.00 a,b	32.80 b	36.44 *
	Mean	42.30	39.33	39.37	40.34
total	2017	44.54 a	42.69 a	45.71 a	44.31 *
	2018	38.95 a	37.57 a	33.46 b	36.66 *
	Mean	41.75	40.13	39.59	40.49

A, B—values followed by the same letters indicate cultivars which differ insignificantly at $p < 0.05$. a, b—values followed by the same letters indicate cultivars which differ insignificantly in a given year at $p \leq 0.05$. Means for years followed by an asterisk (*—in rows) differ significantly at $p \leq 0.05$.

The percentage share of nitrogen taken up by soybean from fertiliser and soil was significantly affected by the study years (Tables 7 and 8) and was higher in 2017 than 2018. Regardless of study years, the percentage of nitrogen derived from fertiliser and soil and accumulated in the roots and post-harvest residues of soybean cultivars was similar. For roots, the values related to accumulation from fertiliser ranged from 12.09% for cv. SG Anser to 13.7% for cv. Abelina. When accumulation from soil was concerned, the mean value was 41.43% (over 44.73% or cv. Abelina, 39.18% for cv. SG Anser and about 40.38% for cv. Merlin). Accumulation of nitrogen from fertiliser in soybean post-harvest residues averaged about 12% (12.30% for cv. Abelina, 11.72% for cv. SG Anser and 12.44% for cv. Merlin), with the average value for accumulation from soil reserves exceeding 48% (48.70% for cv. Abelina, 46.60% for cv. SG Anser and 49.50% for cv. Merlin).

Table 7. Percentage of nitrogen taken up by soybean from fertiliser, %.

Plant Part	Cultivar				Mean
	Year	Abelina	SG Anser	Merlin	
roots	2017	14.40	13.30	12.90	13.53 *
	2018	13.02	10.89	11.83	11.91 *
	Mean	13.71	12.09	12.37	12.72
post-harvest residues	2017	12.90	11.93	12.60	12.48 *
	2018	11.69	11.51	12.29	11.83 *
	Mean	12.30	11.72	12.44	12.15
seeds	2017	8.57 a	9.10 a	8.43 a	8.70 *
	2018	7.56 b	7.88 a,b	8.40 a	7.95 *
	Mean	8.06	8.49	8.42	8.32
total	2017	9.50	9.70	9.50	9.57 *
	2018	8.37	8.56	9.12	8.68 *
	Mean	8.93	9.13	9.31	9.12

a, b—values followed by the same letters indicate cultivars which differ insignificantly in a given year at $p \leq 0.05$. Means for years followed by an asterisk (*—in rows) differ significantly at $p \leq 0.05$.

Table 8. Percentage of nitrogen taken up by soybean from soil reserves, %.

Plant Part	Cultivar				Mean
	Year	Abelina	SG Anser	Merlin	
roots	2017	40.77	37.66	36.53	38.32 *
	2018	48.69	40.70	44.24	44.54 *
	Mean	44.73	39.18	40.38	41.43
post-harvest residues	2017	45.21	41.82	44.16	43.73 *
	2018	52.19	51.39	54.85	52.81 *
	Mean	48.70	46.60	49.50	48.27
seeds	2017	46.35 a	49.23 a	45.63 a	47.07 *
	2018	52.92 b	55.13 a,b	58.80 a	55.62 *
	Mean	49.63	52.18	52.21	51.34
total	2017	45.97 a	47.61 a	44.79 a	46.12 *
	2018	52.68 b	53.88 a,b	57.42 a	54.66 *
	Mean	49.32	50.74	51.11	50.39

a, b—values followed by the same letters indicate cultivars which differ insignificantly in a given year at $p \leq 0.05$. Means for years followed by an asterisk (*—in rows) differ significantly at $p \leq 0.05$.

The percentage of nitrogen taken up from fertiliser and soil stores in the seeds of test cultivars was affected by the study years. The percentage of nitrogen derived from the atmosphere and soil reserves in test soybean cultivars was similar in 2017. In 2018, values for accumulation of nitrogen acquired from fertiliser and soil were lower for cv. Abelina than cv. Merlin, with the respective percentages being 7.56% and 49.64% for cv. Abelina, and 8.40% and 52.23% for cv. Merlin (Tables 7 and 8).

4. Discussion

The study was conducted to ascertain the amount of nitrogen taken up by three cultivars of genetically unmodified soybean obtained from various sources, including the quantity of this macronutrient removed from the field with seeds, and taking into account the amount remaining in post-harvest residues for the following crop. The impulse to undertake the work related to soybean cultivation under European conditions was the fact that at present in Poland, as in the whole of Europe, much stress is placed on increased production of own feed protein and reduction due to this, of imports of soybean meal produced from genetically modified plants.

Soybean cultivation may provide additional benefits associated with limited application of mineral fertilisers which increase yields but also contribute to environment degradation [32]. Post-harvest soybean plant parts may be a nitrogen source for other plants grown in succession [9,33,34] so it is important to determine the amount of nitrogen they accumulate, in particular atmospheric nitrogen. The nitrogen amount left (for the following crop) by individual soybean plant parts depends on their yield. In the experiment reported here, the yield of dry matter of roots, post-harvest residues and seeds was affected by weather conditions. Compared with 2017, a lower yield of these parts was produced by plants grown in 2018 when temperatures were higher, and precipitation was lower (particularly during the seed germination stage). In the study, the dry matter of above-ground plant parts (post-harvest residues) obtained in 2018 was 35% lower compared with 2017. Literature reports indicate that drought is one of the factors which hinder soybean development and yielding [35]. Water shortages lead to reduced plant height [36], particularly when they occur at the stage of shoot formation. Newark [37] and El Kheir et al. [38] have found that insufficient precipitation at this stage may reduce plant height by about 30% to 70% [39]. In the present work, mean root mass developed by cultivars was lower in 2018 when, in June, precipitation exceeded 70 mm and plants did not have to develop an extensive rooting system to increase water supply from the soil. According to Huck et al. [40] and Hirasawa et al. [41], lack of rainfall results in an increase in root matter in the plant. Liu et al. [42] have pointed to a relationship between various root characteristics, including dry matter and resistance to drought. They have demonstrated that for soybean, draught at later stages of vegetative development results in substantial growth of roots, in particular in deeper soil strata. It was also observed that soybean plants produced lower seed mass in 2018 than in 2017, which was probably due to high temperatures and insufficient precipitation. Mandić et al. [39,43] and Ghassemi-Golezani and Lotfi [44] have found that drought occurring during the reproductive period (July–August) causes seed dieback, reduces seed size, shortens the period of seed fill and, as a result, reduces seed yield. According to Purcell et al. [45] and Sinclair et al. [46], nitrogen content in plants may decline due to insufficient precipitation (water stress) and increased temperatures, which was also confirmed in the work discussed here. In 2018, which was hot and dry, soybean plants contained less nitrogen than in 2017, regardless of the cultivar.

Both the amount of BNF-originating nitrogen and its percentage were affected by growing conditions. In the dry and warmer 2018, the whole soybean biomass accumulated over 36 kg $N{\cdot}ha^{-1}$, which was less than in 2017 (65.32 $N{\cdot}ha^{-1}$). Meteorological conditions were a significant factor in a model for predicting the amount of BNF in soybean plants formulated by Collino et al. [47]. Wysokiński et al. [19] as well as Divito and Sadras [48] have pointed to water shortages in soil as a factor limiting the percentage and amount of BNF in legumes. Giunet et al. [49] claim that the effect of water shortages on the quantity of fixed nitrogen depends on plant species, the sensitive species including common bean, soybean and vetch. In contrast, chickpea tends to be resistant to stress conditions. Climate conditions can also shape non-symbiotic microbial communities, that can play a role in N availability in the soil throughout the plant cycle, and precipitation patterns and soil microbial diversity induce a stimulation of root growth and development [50].

In the present work, the dry mass of roots, post-harvest residues and seeds was cultivar-related. In addition to environmental factors, the genetic factor exerts the strongest influence on yields [43,51,52]. Lower values of the mass of all the parts of cv. Merlin indicate that the cultivar more poorly adjusted to changing environmental conditions. Cv. Merlin is slightly older than SG Anser or Abelina. Many studies have confirmed that older cultivars find it more difficult to adjust to environmental conditions and stress-related factors, which results in their poorer growth and yield performance [53–56]. In the present work, the mean nitrogen content in seeds, roots and post-harvest residues was around 45, 11.0 and 5.5 $g{\cdot}kg^{-1}$, respectively. The results correspond to findings reported by Kahira et al. [57], who demonstrated that nitrogen content was affected by cultivation place and was the highest in grain, ranging from 5.87% to 6.15%, and the lowest in roots, where it ranged from 0.89% to 1.18%.

Zangh et al. [58] have found that nitrogen content was higher in shoots versus roots, which they believe indicated that the nitrogen was transported to the above-ground parts to develop shoots.

The accumulation of the isotope ^{15}N by various plant parts differed substantially. Larger amounts of the isotope were recorded in plant roots than post-harvest residues and seeds, which may indicate that these parts contained more nitrogen [19]. Also, Kihara et al. [57] observed higher ^{15}N contents in roots than in other parts (stems and leaves, pods, grains). In their study, Yoneyama et al. [59] concluded that differences in the amount of isotope taken up by various soybean parts may have been due to different forms of ^{15}N compounds transported in the xylem. Soybean plants transport nitrogen from roots to other parts as ureides (allantoin and allantoic acid). The NO_3^- they have absorbed may be transported to the shoot as nitrate and asparagine, whereas absorbed $NH^+{}_4$ may be converted to glutamine and asparagine in the roots.

Soybean absorbs nitrogen from three alternative sources: biological nitrogen fixation (BNF), absorption from soil and from fertilisers. For soybean, the highest percentage of absorbed nitrogen, from 40% to 80%, is atmospheric nitrogen (BNF) [6]. Depending on the cultivation region, this amount may be different, as indicated by research conducted in various countries. In Argentina, the percentage of BNF-related nitrogen was estimated to range from 26% to 71% [60–62], whereas in North America and Brazil, the value exceeded 75% [63,64]. Similar results of their studies were reported by Kihara et al. [57], Santachiara et al. [22], Collino et al. [47] and Zhang et al. [58]. The research discussed here demonstrated that the total soybean biomass took up about 40% nitrogen from the atmosphere, regardless of the study year or cultivar. Cv. SG Anser had the highest share of atmospheric nitrogen in roots and post-harvest residues, and cv. Abelina in its seeds. According to Santachiara et al. [22], the influence of cultivar may account for as much as 90% of the variation in the total nitrogen accumulation, whereas between-cultivar differences may also result from the source of N absorption (BNF or from soil). The authors demonstrated that two-thirds of cultivars (out of 70 examined cultivars) accumulated more nitrogen take up from soil than the atmosphere. Such a situation was observed in the work reported here, which indicates that soil reserves were the main source of nitrogen absorbed by the whole biomass of test cultivars. A substantial amount of nitrogen absorbed from soil reserves may result from a long soybean growing season and an occurrence of conditions conducive to organic matter mineralisation in soil during this period (applied mineral nitrogen, high temperature, optimum moisture-related conditions). A high percentage of nitrogen derived from soil reserves in the total soybean mass may also be related to the fact that more nitrogen in seeds of the test cultivars had been taken up from soil than the atmosphere. Differences in nitrogen absorption between cultivars growing under the same conditions may also be related to genetic effects or physiological limitations [65,66].

The quantity of nitrogen taken up from fertiliser was lower compared with N amounts acquired from soil reserves and the atmosphere, which was a result of using a low mineral nitrogen rate in the experiment. Such a nitrogen rate did not restrict the nitrogen fixation process, which made it possible to calculate the amount of the macronutrient absorbed by soybean from different sources. High concentrations of mineral nitrogen forms in soil hinder the process of elemental nitrogen reduction by rhizobia, thus limiting the activity of enzymes participating in this process and the development of root nodules [67,68]. Giunet et al. [49] observed inhibition of symbiotic nitrogen fixation by inorganic N. In contrast, research conducted by LeMenza et al. [69] has shown that soybean, when grown in conditions which allow the crop to produce yields higher than 2.5 $Mg \cdot ha^{-1}$, may positively respond to nitrogen fertilisation.

5. Conclusions

In the study reported here, a significant effect of soybean cultivars and study years on the amount of nitrogen taken up from the atmosphere, soil reserves and mineral fertiliser was confirmed. When all the sources were concerned, cv. Merlin accumulated less nitrogen in the whole biomass, post-harvest residues and seeds than cv. SG Anser or Abelina. In the year characterised by favourable precipitation and thermal conditions (2017), soybean took up more nitrogen from all the sources than in 2018.

The amount of nitrogen removed from the field with seeds averaged 98.608 kg N·ha^{-1}, with the respective amounts for the atmosphere, soil reserves and mineral fertiliser sources being 40.43, 49.89 and 8.29 kg N·ha^{-1}. The quantity of nitrogen absorbed from all the sources (atmosphere, fertiliser and soil reserves) and introduced into the soil with post-harvest residues and roots exceeded 29 kg N·kg^{-1}. The actual soil enrichment with atmospheric nitrogen averaged 10.53 kg N·ha^{-1}, with the respective values for cv. Merlin, Abelina and SG Anser being 9.57, 10.41 and 11.60 kg N·ha^{-1}. The research demonstrated that soil reserves and the atmosphere were the dominant sources of nitrogen for soybean. The respective shares of nitrogen taken up from soil reserves, the atmosphere and mineral fertiliser were 50.4%, 40.5% and 9.1%.

Research on symbiotic fixation of atmospheric nitrogen by legumes is of importance to agriculture, all the more so because the plants display marked species variation in this respect. Increasing demand for non-GMO soybean in Europe and a steadily growing land area devoted to the crop explain the need to carry out this type of research. It is difficult to determine the actual amount of biologically fixed nitrogen due to the lack of precise parameters describing this process, which is also affected by habitat conditions such as weather conditions, soil pH, mineral fertilisation, etc. Thus, it is necessary to conduct further research based on precise modelling, including the conditions of plant growth and development. It is of particular importance to accurately determine the availability for the following crops of nitrogen introduced into the soil with soybean post-harvest residues and taken up from the atmosphere.

Author Contributions: Conceptualization, A.W., K.R. and E.R.; Methodology, A.W. and E.R., software, K.R.; writing—original draft preparation, E.R., K.R. and A.W.; writing—review and editing, E.R., K.R. and A.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

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

References

1. Van Veen, J. Nitrogen: Recent developments in related microbial processes. In *Biological Resource Management Connecting Science and Policy*; Balázs, E., Galante, E., Lynch, J.M., Schepers, J.S., Toutant, J.P., Werner, D., Werry, P.T.J., Eds.; Springer: Berlin/Heidelberg, Germany, 2000; pp. 71–80. [CrossRef]
2. Hardoim, P.R.; van Overbeek, L.S.; Berg, G.; Pirttilä, A.M.; Compant, S.; Campisano, A.; Döring, M.; Sessitsch, A. The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial Endophytes. *Microbiol. Mol. Biol. Rev.* **2015**, *79*, 293–320. [CrossRef]
3. Martyniuk, S. Biological fixation of N_2, symbiotic bacteria of legumes in Polish soils and estimation of their numbers. *Pol. J. Agron.* **2019**, *38*, 52–65. (In Polish).
4. Florek, J.; Czerwińska-Kayzer, M.; Jerzak, A. Current state of production and use of leguminous crops. *Fragm. Agron.* **2012**, *29*, 45–55.
5. Willems, A. The taxonomy of rhizobia: An overview. *Plant Soil* **2006**, *287*, 3–14. [CrossRef]
6. Salvagiotti, F.; Cassman, K.G.; Specht, J.E.; Walters, D.T.; Weiss, A.; Dobermann, A. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crops Res.* **2008**, *108*, 1–13. [CrossRef]
7. Salvagiotti, F.; Specht, J.E.; Cassaman, K.G.; Walters, D.T.; Weiss, A.; Dobermann, A. Growth and nitrogen fixation in high-yielding soybean: Impact of nitrogen fertilization. *Agron. J.* **2009**, *101*, 958–970. [CrossRef]
8. Martyniuk, S. The importance of biological fixation of atmospheric nitrogen in ecological agriculture. *J. Appl. Agric. Eng.* **2008**, *53*, 9–14. (In Polish).
9. Singh, M.; Wanjari, R.H.; Agrawal, B.K.; Sharma, P. Biological N2 fixation in soybean and contribution to soil in a 40-year-old experiment on Alfisols of Ranchi. *J. Indian Soc. Soil Sci.* **2014**, *62*, 56–61.
10. Martyniuk, S. 2012. Scientific and practical aspects of legumes symbiosis with root-nodule bacteria. *Pol. J. Agron.* **2012**, *9*, 17–22. (In Polish).
11. Graham, P.H.; Vance, C.P. Legumes importance and constraints to greater utilization to greater utilization. *Plant Physiol.* **2003**, *131*, 872–877. [CrossRef]

12. Sanginga, N. Role of biological nitrogen fixation in legume based cropping systems; a case study of West Africa farming systems. *Plant Soil* **2003**, *252*, 25–39. [CrossRef]
13. Van, V.D.; Franke, A.C.; Giller, K.E. Participatory research to close the soybean yield gap on smallholder farmers in Malawi. *Exp. Agric.* **2017**, *53*, 396–415. [CrossRef]
14. Ciampitti, I.A.; Salvagiotti, F. New insights into soybean biological nitrogen fixation. *Agron. J.* **2008**, *110*, 1185–1196. [CrossRef]
15. Temperly, R.J.; Borges, R. Tillage and crop rotation impact on soybean grain yield and composition. *Agron. J.* **2006**, *98*, 999–1004. [CrossRef]
16. Bellaloui, N.; Bruns, H.A.; Gillen, A.M.; Abbas, H.K.; Zablotowicz, R.M.; Mengistu, A.; Paris, R.L. Soybean seed protein, oil, fatty acid and mineral composition as influence by soybean corn rotation. *Agric. Sci.* **2010**, *1*, 102–109. [CrossRef]
17. Bellaloui, N.; Stetina, S.R.; Molin, W.T. Soybean seed nutrition as affected by cotton, wheat, and follow rotation. *Food Nutr. Sci.* **2014**, *5*, 1605–1619. [CrossRef]
18. Bellaloui, N.; Smith, J.R.; Gillen, A.M.; Ray, J.D. Effect of maturity on seed sugars as measured on near-isogenic soybean (*Glycine max*) Lines. *Crop Sci.* **2010**, *50*, 1978–1987. [CrossRef]
19. Wysokiński, A.; Kalembasa, S.; Symanowicz, B. Dynamic of nitrogen accumulation by pea (*Pisum Sativum* L.) from different sources. *Fragm. Agron.* **2013**, *30*, 162–169. (In Polish). Available online: http://www.pta.iung.pulawy.pl/wydawni (accessed on 12 June 2020).
20. Wysokiński, A.; Kuziemska, B. The sources of nitrogen for yellow lupine and spring triticale in their intercropping. *Plant Soil Environ.* **2019**, *65*, 145–151. [CrossRef]
21. Rotundo, J.L.; Borrás, L.; de Bruin, J.; Pedersen, P. Soybean nitrogen uptake and utilization in Argentina and united state cultivars. *Crop Sci.* **2014**, *54*, 1153–1165. [CrossRef]
22. Santachiara, G.; Borrás, L.; Salvagiotti, F.; Gerde, J.A.; Rotundo, J.L. Relative importance of biological nitrogen fixation and mineral uptake in high yielding soybean cultivars. *Plant Soil* **2017**, *418*, 191–203. [CrossRef]
23. Cluskey, J.M.; Grimsrud, K.M.; Ouchi, H.; Wahl, T.I. Consumer response to genetically modified food products in Japan. *Agric. Resour. Econ. Rev.* **2003**, *32*, 222–231. [CrossRef]
24. Twardowski, T.; Małyska, A. Social and legal determinants for the marketing of GM products in Poland. *New Biotech.* **2012**, *29*, 249–254. [CrossRef]
25. Garrett, R.D.; Rueda, X.; Lambin, E.F. Globalization's unexpected impact on soybean production in South America: Linkages between preferences for non-genetically modified crops, eco-certifications, and land use. *Environ. Res. Lett.* **2014**, *8*, 1–11. [CrossRef]
26. Alves, B.J.; Boddey, R.M.; Urquiaga, S. The success of BNF in soybean in Brazil. *Plant Soil.* **2003**, *252*, 1–9. [CrossRef]
27. Márton, L. Biological n-fixation (BNF) under different N-fertilizer input (NFI) on soya yield. In *Advanced Soil Science Theory and Practice*; Hungary-Institute for Soil Science and Agricultural Chemistry Centre for Agricultural Researches of the Hungarian Academy of Sciences: Budapest, Hungary, 2006; pp. 99–104.
28. László, M. Biological N2-FIXATION and Mineral N-Fertilization Effects on soybean (*Glicine max* L. Merr.). In Proceedings of the Yield Under Temperate Climate Conditions EGU General Assembly, Vienna, Austria, 19–24 April 2009; p. 1374.
29. World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil. In *World Soil Resources Reports 106; Field Experiment*; Food and Agriculture Organization: Rome, Italy, 2014.
30. Azam, F.; Farooq, S. An appraisal of methods for measuring symbiotic nitrogen fixation in legumes. *Pak. J. Biol. Sci.* **2003**, *6*, 1631–1640. [CrossRef]
31. Kalembasa, S.; Wysokiński, A.; Kalembasa, D. Quantitative assessment of the process of biological nitrogen reduction by yellow lupine (Lupinus luteus L.). *Acta Sci. Pol. Agric.* **2014**, *13*, 5–20.
32. Ahmed, A.; Aftab, S.; Hussain, S.; Nazir Cheema, H.; Liu, W.; Yang, F.; Yang, W. Nutrient accumulation and distribution assessment in response to potassium application under maize–soybean intercropping system. *Agronomy* **2020**, *10*, 725. [CrossRef]
33. Graham, P.H.; Vance, C.P. Nitrogen fixation in perspective: An overview of research and extension needs. *Field Crops Res.* **2000**, *65*, 93–106. [CrossRef]
34. Księżak, J.; Staniak, M.; Bojarszczuk, J. The regional differentiation of legumes cropping area in Poland between 2001 and 2007. *Pol. J. Agron.* **2009**, *1*, 25–31.

35. Manavalan, L.P.; Guttikonda, S.K.; Tran, L.S.P.; Nguyen, H.T. Physiological and molecular approaches to improve drought resistance in Soybean. *Plant Cell Physiol.* **2009**, *50*, 1260–1276. [CrossRef] [PubMed]
36. Basal, O.; Szabó, A. the combined effect of drought stress and nitrogen fertilization on soybean. *Agronomy* **2020**, *10*, 384. [CrossRef]
37. Newark, D.E. Plant growth with limited water-report. *Energy Res. Abstr.* **1991**, *17*, 1812.
38. Kheir, M.S.E.; Kandil, S.A.; Mekki, B.B. Physiological response of two soybean cultivars grown under water stress conditions as aected by CCC treatment. *J. Physiol. Sci.* **1994**, *18*, 179–200.
39. Mandić, V.; Đorđević, S.; Bijelić, Z.; Krnjaja, V.; Pantelić, V.; Simić, A.; Dragičević, V. Agronomic responses of soybean genotypes to starter nitrogen fertilizer rate. *Agronomy* **2020**, *10*, 535. [CrossRef]
40. Huck, M.G.; Ishihara, K.; Peterson, C.M.; Ushijima, T. Soybean adaptation to water stress at selected stages of growth. *Plant Physiol.* **1983**, *73*, 422–427. [CrossRef]
41. Hirasawa, T.; Tanaka, K.; Miyamoto, D.; Takei, M.; Ishihara, K. Effects of pre-flowering moisture deficits on dry matter production and ecophysiological characteristics in soybean plants under drought conditions during grain fi lling. *Jpn. J. Crop Sci.* **1994**, *63*, 721–730. [CrossRef]
42. Liu, F.; Anderson, M.N.; Jacobson, S.E.; Jensen, C.R. Stomatal control and water use efficiency of soybean (Glycine max L. Merr.) during progressive soil drying. *Environ. Exp. Bot.* **2005**, *54*, 33–40. [CrossRef]
43. Mandić, V.; Simić, A.; Krnjaja, V.; Bijelić, Z.; Tomić, Z.; Stanojković, A.; Muslić, D.R. Effect of foliar fertilization on soybean grain yield. *Biotechnol. Anim. Husb.* **2015**, *31*, 133–143. [CrossRef]
44. Ghassemi-Golezani, K.; Lotfi, R. Influence of water stress and pod position on oil and protein accumulation in soybean grains. *Int. J. Plant Prod.* **2013**, *4*, 2341–2345.
45. Purcell, L.C.; Serraj, R.; Sinclair, T.R.; De, A. Soybean N2 fixation estimates, ureide concentration, and yield responses to drought. *Crop Sci.* **2003**, *44*, 484–492. [CrossRef]
46. Sinclair, T.R.; Messina, C.D.; Beatty, A.; Samples, M. Assessment across the United States of the benefits of altered soybean drought traits. *Agron. J.* **2010**, *102*, 475–482. [CrossRef]
47. Collino, D.J.; Salvagiotti, F.; Perticari, A.; Piccinetti, C.; Ovando, G.; Urquiaga, S.; Racca, R.W. Biological nitrogen fixation in soybean in Argentina: Relationships with crop, soil, and meteorological factors. *Plant Soil* **2015**, *392*, 239–252. [CrossRef]
48. Divito, G.A.; Sadras, V.O. How do phosphorus, potassium and sulphur affect plant growth and biological nitrogen fixation in crop and pasture legumes? A meta-analysis. *Field Crop Res.* **2014**, *156*, 161–171. [CrossRef]
49. Guinet, M.; Nicolardot, B.; Revellin, C.; Durey, V.; Carlsson, G.; Voisin, A. Comparative effect of inorganic N on plant growth and N2 fixation of ten legume crops: Towards a better understanding of the differential response among species. *Plant Soil* **2018**, *432*, 207–227. [CrossRef]
50. Prudent, M.; Dequiedt, S.; Sorin, C.; Girodet, S.; Nowak, V.; Duc, G.; Salon, C.; Maron, P. The diversity of soil microbial communities matters when legumes face drought. *Plant Cell Environ.* **2020**, 1–13. [CrossRef]
51. Parente, T.L.; Lazarini, E.; Caioni, S.; Pivetta, R.S.; Souza, L.G.M.; Bossolani, J.W. Adubação nitrogenada em genótipos de soja associada à inoculação em semeadura direta no Cerrado. *Rev. Bras. Ciênc. Agrár.* **2015**, *10*, 249–255. [CrossRef]
52. Soares, I.; de Rezende, P.; Bruzi, A.; Zuffo, A.; Zambiazzi, E.; Fronza, V.; Teixeira, C. Interaction between soybean cultivars and seed density. *Am. J. Plant. Sci.* **2015**, *6*, 1425–1434. [CrossRef]
53. He, J.; Du, Y.L.; Wang, T.; Turner, N.C.; Yang, R.P.; Jin, Y.; Xi, Y.; Zhang, C.; Cui, T.; Fang, X.W. Conserved water use improves the yield performance of soybean (Glycine max (L. Merr.)) under drought. *Agric. Water Manag.* **2017**, *179*, 236–245. [CrossRef]
54. He, J.; Du, Y.L.; Wang, T.; Turner, N.C.; Xi, Y.; Li, F.M. Old and new cultivars of soya bean (*Glycine max* L.) subjected to soil drying differ in abscisic acid accumulation, water relations characteristics and yield. *J. Agron. Crop Sci.* **2016**, *202*, 372–383. [CrossRef]
55. He, J.; Jin, Y.; Turner, N.C.; Li, F.M. Irrigation during flowering improves subsoil water uptake and grain yield in rainfed soybean. *Agronomy* **2020**, *10*, 120. [CrossRef]
56. Gao, X.-B.; Guo, C.; Li, F.-M.; Li, M.; He, J. High soybean yield and drought adaptation being associated with canopy architecture, Water uptake, and root traits. *Agronomy* **2020**, *10*, 608. [CrossRef]
57. Kihara, J.; Martius, C.; Bationo, A.; Vlek, P.L.G. Effects of tillage and crop residue application on soybean nitrogen fixation in a tropical ferralsol. *Agriculture* **2011**, *1*, 22–37. [CrossRef]

58. Zhang, R.; Wang, C.; Teng, W.; Wang, J.; Lyu, X.; Dong, S.; Kang, S.; Gong, Z.; Ma, C. Accumulation and distribution of fertilizer nitrogen and nodule-fixed nitrogen in soybeans with dual root systems. *Agronomy* **2020**, *10*, 397. [CrossRef]
59. Yoneyama, T.; Ito, O.; Engelaar, W.M.H.G. Uptake, metabolism and distribution of nitrogen in crop plants traced by enriched and natural 15N: Progress over the last 30 years. *Phytochem. Rev.* **2003**, *2*, 121–132. [CrossRef]
60. Di Ciocco, C.; Alvarez, R.; Andrada, Y.; Momo, F. Nitrogen balance in a soybean crop. *Cienc Suelo* **2004**, *22*, 48–51. [CrossRef]
61. Di Ciocco, C.; Coviella, C.; Coviella, C.; Penon, E.; Diaz-Zorita, M.; Lopez, S.; Lopez, S. Biological fixation of nitrogen and N balance in soybean crops in the pampas region. *Span. J. Agric. Res.* **2008**, *6*, 114–119. [CrossRef]
62. Di Ciocco, C.; Penón, E.; Coviella, C.; Lopez, S.; Diaz-Zorita, M.; Momo, F.; Alvarez, R. Nitrogen fixation by soybean in the Pampas: Relationship between yield and soil nitrogen balance. *Agrochimica* **2011**, *55*, 305–313.
63. Herridge, D.; Peoples, M.; Boddey, R. Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil* **2008**, *311*, 1–18. [CrossRef]
64. Hungria, M.; Franchini, J.C.; Campo, R.J.; Crispino, C.C.; Moraes, J.Z.; Sibaldelli, R.N.R.; Mendes, I.C.; Arihara, J. Nitrogen nutrition of soybean in Brazil: Contributions of biological N2 fixation and N fertilizer to grain yield. *Can J. Plant Sci.* **2006**, *86*, 927–939. [CrossRef]
65. Santos, M.A.; Geraldi, I.O.; Garcia, A.A.F.; Bortolatto, N.; Schiavon, A.; Hungria, M. Mapping of QTLs associated with biological nitrogen fixation traits in soybean. *Hereditas* **2013**, *150*, 17–25. [CrossRef] [PubMed]
66. Hwang, S.; Ray, J.D.; Cregan, P.B.; King, C.A.; Davies, M.K.; Purcell, L.C. Genetics and mapping of quantitative traits for nodule number, weight, and size in soybean (*Glycine max* L. Merr.). *Euphytica* **2014**, *195*, 419–434. [CrossRef]
67. Ohyama, T.; Tewari, K.; Ishikawa, S.; Tanaka, K.; Kamiyama, S.; Ono, Y.; Hatanao, S.; Ohtake, N.; Sueyoushi, K.; Hasegawa, H.; et al. Role of nitrogen on growth and seed yield of soybean and a new fertilization technique to promote nitrogen fixation and seed yield. In *Soybean: The Basis of Yield, Biomass and Productivity*; Kasai, M., Ed.; InTech Croatia: Rijeka, Croatia, 2017; pp. 153–185. [CrossRef]
68. Gałęzewski, L.; Jaskulska, I.; Wilczewski, E.; Wenda-Piesik, A. Response of yellow lupine to the proximity of other plants and unplanted path in strip intercropping. *Agriculture* **2020**, *10*, 285. [CrossRef]
69. LaMenza, N.C.; Monzon, J.P.; Soecht, J.E.; Grassini, P. 2017. Is soybean yield limited by nitrogen supply? *Field Crop Res.* **2017**, *231*, 204–212. [CrossRef]