



Article The Nitrogen Fixation and Yielding of Pea in Different Soil Tillage Systems

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Abstract: The field experiment was carried out in 2013 and 2014 as part of a long-term experiment to test the influence of different soil tillage systems (conventional, reduced, direct sowing) on (a) the yield of summer pea (Pisum sativum L.) and (b) the amount of biologically reduced atmospheric nitrogen (N), which was determined using the ¹⁵N dilution method (ID¹⁵N). Spring barley was used as a reference plant. Climatic conditions did not have a significant influence on the yield of pea seeds (mean value 4.56 t ha⁻¹), post-harvest residues (3.76 t ha⁻¹) and total biomass (8.33 t ha⁻¹). Soil tillage system was found to have a significant impact on all components of the pea yield in years of experiment, with the highest average seed yield values observed with the conventional system (5.19 t ha^{-1}) and significantly lower values observed with reduced tillage (4.34 t ha}{-1}) and direct sowing (4.17 t ha^{-1}) . The content of total N was greatest in the pea biomass that was harvested in the conventional system (202 kg ha⁻¹) and lowest with direct sowing (155 kg ha⁻¹). Nitrogen accumulated in the pea seeds from three sources: atmosphere (mean value 35.2%), fertilizers (6.8%) and soil (57.9%), equating to 48.6, 9.9, and 85.4 kg ha^{-1} , respectively. Soil tillage system was found to have a significant impact on the amount of N that was fixed from the atmosphere by the peas: 17.7% in conventional tillage, 37.9 in reduced system and 50.2% in direct sowing, which equates to a harvested seed yield of 28.9, 52.0 and 64.4 kg ha⁻¹, respectively. In the post-harvest residues, the amount of N fixed from the atmosphere by the pea crop was also modified by the soil tillage system in range: 20.2% in conventional tillage and 32.6% in direct sowing (which equates to 7.4 and 8.5 kg N ha^{-1} , respectively), but the difference was not significant.

Keywords: ¹⁵N isotope; legumes; biological nitrogen fixation; nitrogen balance; yield

1. Introduction

Pea (*Pisum sativum* L.) is a leguminous plant that is cultivated globally because of its nutritional value [1–3] and its beneficial effect as a pre-crop in crop rotations [4,5]. Despite the obvious advantages, the area under pea cultivation globally remains low and has decreased in recent years [6]. This is mainly caused by low profitability and fluctuating yields, produced by biotic and abiotic factors. However, due to symbiosis with *Rhizobium*, pea can fix atmospheric nitrogen (N), which can also help decrease costs for the grower through reduced inorganic applications, although some studies [7,8] recommend the application of a low dose of N (in the form of mineral fertilizer) at sowing time or at the beginning of pea growth (so-called starting dose).

In recent years, the substitution of soil ploughing by reduced or no-till soil cultivation systems has been employed [9-13]. The use of these simplified systems can decrease the



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). expenditure on energy and labor [9] and can have positive effects on the soil environment, such as increased carbon (C) content and microbial activity [10].

Gajda et al. [14] investigated the influence of three soil tillage systems: conventional, reduced and no-till on the microbial C biomass and N content, microbial respiration rate, and dehydrogenase and arylsulfatase activity. After eight years, the reduced tillage system was observed to enhance soil biological activity by an average 15–40% in comparison to the conventional tillage system. Moreover, Małecka-Jankowaik et al. [8] stated that the long-term application of reduced tillage and direct sowing was favorable for increasing the activity of soil enzymes.

However, the advantages of no-till systems can be offset by a large weed population, which has a negative influence on legume yield. In this case, mechanical or chemical methods must be used to reduce or eliminate the weed population [10,15].

The concept of sustainable crop production seeks to determine new approaches to increase the cultivation of legumes in crop rotations and thereby maximize biological N fixation. Moreover, determination of the most appropriate method to quantify the amount of N that is biologically fixed from the atmosphere is critical to calculate the N balance (and fertilizer requirement) of cultivated plants. The ¹⁵N dilution isotope technique has been widely used to measure the amount of biological N fixation. This method is useful because it enables the amount of atmospheric N fixed by the legumes, and N uptake from the soil and fertilizer by the plants to be quantified.

The background of the hypothesis of our research was that long-term tillage systems can influence in different way on the process of nitrogen fixation by legume and its yield. The aim of this study was to investigate the long-term effect of different tillage systems on biological N reduction by pea plants as determined by the isotope dilution method (ID¹⁵N).

2. Materials and Methods

2.1. Site Description

The field experiment was carried out during 2013 and 2014 as part of a long-term field experiment that commenced in 1999 at the Agricultural Experimental Station in Brody (52°26′ N, 16°17′ E), which investigated the impact of different soil tillage systems (conventional, reduced, direct sowing) on crop yields. Table 1 shows meteorological conditions during the vegetation period in 2013–2014.

Table 1. Air temperature (°C) and rainfall sum (mm) during the vegetation period in 2013–2014. Data source: Meteorological Station, Brody, Poland.

	Mean Air Temperature (°C)					Rainfall Sum (mm)				
Day	Month/Year									
of Month	April	May	June	July	\overline{x}	Aprrl	May	June	July	Σ
					20	013				
1st-10th	1.3	15.0	16.1	19.6	13.0	1.4	16.2	16.8	5.0	39.1
11th-20th	10.9	15.8	19.4	18.6	16.1	9.6	1.3	0.3	0.0	11.2
21st-30th/31st	11.9	12.5	16.4	22.3	15.8	4.4	52.3	108.2	62.3	227.2
$\overline{x} * / \sum **$	8.0 *	14.4	17.3	20.1	14.9	15.4 **	69.8	125.3	67.3	277.8
				20	014					
1st-10th	8.8	10.1	17.5	20.5	14.2	12.5	14.5	3.3	31.0	61.3
11th-20th	8.7	12.6	16.0	21.7	14.8	10.5	21.4	24.0	7.8	63.7
21st-30th/31st	13.9	16.5	14.8	22.2	16.9	23.3	37.6	14.7	44.3	119.9
$\overline{x} * / \sum **$	10.5 *	13.1	16.1	21.5	15.3	46.3 **	73.5	42.0	83.1	244.9
Long term value	8.0	13.2	16.5	18.2	18.6	37.2	57.1	64.1	81.2	239.6

* Mean monthly air temperature (°C) ** monthly rainfall sum (mm).

The soil at the study site is classified as Albic Luvisoil, and as Typic Hapludalf [16]. Following granulometric composition analysis, the field soil was ascertained to be sandy

clay loam. The granulometric composition and some chemical properties of the field soil are presented in Tables 2 and 3. Soil samples were taken from the top layer of soil profile up to 20 cm depth.

Table 2. Selected physical properties of soil at the study site.

Granulometric Composition Fraction	mm	%
Sand	2.00-0.05	62
Silt	0.05-0.002	29
Clay	≤ 0.002	9

Table 3. Selected chemical properties of soil at the study site.

Chemical Soil Properties				
pH in 1M KCl	6.3			
$C (g kg^{-1})$	10.5			
$N (g kg^{-1})$	0.91			
P (available mg kg $^{-1}$)	147			
K (available mg kg ^{-1})	198			
Mg (available mg kg $^{-1}$)	67			

2.2. Experiment Design and Agronomic Management

The current study was a one-way experiment with completely randomized blocks in three replications with two crops: spring pea var. Tarchalska (investigated plant) and summer barley var. Rubinek (reference plant). During the growth and development stages of the studied plants, agricultural practices were carried out in accordance with established management principles. Sowing and harvest dates depended on species and weather conditions. Seeds and grain were sown in the last week of March: pea at 100 seeds per 1 m² and summer barley at 500 grains per 1 m². The sowing depth in all tillage systems was 4 cm and the row space was 18 cm. In all the tillage systems, plots were drilled with a double disk drill (Great Plains, Solid Stand 100 equipped with a fluted coulter for residue cutting, a double disk for seed placement, and a press wheel (3 m wide), weight of the tractor was 2885 kg). The area of each replication was 24 m² (plot area: 4 m width × 6 m length) where mineral fertilizer (82.5 kg P₂O₅ ha⁻¹, 80 kg K₂O ha⁻¹) was applied in early spring in each year.

After the emergence of the plants, each plot was divided on two treated subplots (area of the subplot 2 m width \times 2 m length = 4 m², the total are of two treated subplots 8 m² per replication), which were established in the middle of the plot of each replication. ¹⁵N fertilizer in the form of ammonium sulfate (¹⁵NH₄)₂SO₄ in solution (containing 37.5 at%¹⁵N) was applied at a rate of 30 kg N ha⁻¹ to the subplots in the pea and spring barley (reference plant) plots. The amount of N applied was considered as a starting dose. A reference crop is a plant that does not biologically fix nitrogen and its only source of nitrogen are soil and fertilizer, by using ¹⁵N it is possible to compare the analyzed features in biomass both studied crops. To evaluate the yield and study the features, the pea and spring barley were harvested from each subplot from area 2 m², where ¹⁵N fertilizer was applied. Yield of seeds/grain plus post-harvest residues (vegetative parts of plants) are considered "total biomass". During the growing season, recommended pesticides were used to target specific insect and plant species.

The experimental design contained three soil tillage systems:

- (1) Conventional tillage: ploughing with a 3-furrow reversible plough was carried out each year to a depth of 25 cm.
- (2) Reduced till in stubble cultivation was carried out annually at autumn (stubble cultivator 2.5 m wide).
- (3) Direct sowing each year into the stubble of the pre-crop with no basic land preparation.

The pea and spring barley crops were harvested at full maturity growth stage (BBCH 90) and samples of the plant materials were ground down to a particle diameter < 0.25 mm. Total N content was determined by the Kjeldahl method. The $^{15}N/^{14}N$ isotope ratio in the samples of both crops were analyzed on an elemental analyzer Flash EA 1112HT (Thermo Scientific, Waltham, MA, USA) coupled with the mass spectrometer Delta V Advantage (Thermo Scientific) in a continuous flow system with helium.

2.4. Calculations

Biological N reduction was calculated using the following formulas provided by Kalembasa et al. [17] including:

- (1) % N derived from the atmosphere % Ndfa = $[1 (at\% {}^{15}Nexcess in pea */at\% {}^{15}Nexcess in spring barley *) \times 100$
- (2) Amount of atmospheric N fixed by the pea (kg ha⁻¹) N fixed from atmosphere (kg ha⁻¹) = (% Ndfa × TN)/100 where: TN = total amount of N in the pea (kg ha⁻¹).
- (3) % N derived from fertilizer % Ndff = (at% ¹⁵Nexcess in pea */at% ¹⁵Nexcess in fertilizer *) × 100
- (4) Amount of N derived from fertilizer (kg ha⁻¹) Ndff (kg ha⁻¹) = (% Ndff \times TN)/100
- (5) % N derived from the soil % Ndfs = 100 (% Ndfa + % Ndff).
- (6) Coefficient of N-utilization (N-use efficiency) from the fertilizer NUE (%) = (Ndff kg ha^{-1}/D kg $ha^{-1}) \times 100$ where: D = dose of N applied in the N fertilizer
- (7) Total amount of ¹⁵N in the biomass of the pea and spring barley plants (kg ha⁻¹) ¹⁵N (kg ha⁻¹) = (at% ¹⁵Nexcess in plant × TN in plant)/100
- (8) % of ¹⁵N uptake = (¹⁵N kg ha⁻¹ in plant/¹⁵N kg ha⁻¹ in fertilizer) × 100 at%¹⁵Nexcess = at% ¹⁵Nexcess in samples (pea, barley or fertilizer) 0.3663

2.5. Statistical Analysis

The results observed during the field experiment and chemical analyzes were statistically calculated with one-factor ANOVA analysis of variance (Statistica PL. Software Statsoft. Tulsa, OK, USA). Significant differences were calculated using Tukey's test at a significance level of p < 0.05. In order to show the existing regularities (correlations) between yield and features of plants average for years of research, a Pearson correlation matrix was determined, which was illustrated using a heatmap.

3. Results and Discussion

The average pea seed yield was 4.56 t ha⁻¹ and there was no observed significant difference between two harvest years, although the yield was slightly higher in 2014 (Table 4). The experimental factor (soil tillage system) resulted in a significantly modified pea seed yield. Both simplified soil tillage systems (reduced and direct sowing) resulted in decreased pea seed yield.

The highest yield (5.19 t ha⁻¹) was found with conventional tillage and the yield was considerably lower in reduced tillage (by 16.4%) and direct sowing (by 19.6%). At the same experimental station in Brody, Małecka-Jankowiak et al. [8] reported that the highest pea yield occurred with reduced tillage, lower yields with conventional tillage and lowest yields with direct sowing. Similar results were presented by Woźniak [18], but Faligowska and Szukała [19] received opposite results. The results reported by Santín-Montanyá et al. [11], which examined the impact of the soil tillage system on pea yield did not show any significant differences.

These differences between studies are probably caused by several factors, such as the soil biological and microbiological communities and the physical properties of the soil [20,21]. Moreover, the amount and distribution of rainfall are likely to have a huge influence on pea seed yield, although in the growing season of 2013, rainfall at our study site was higher than in 2014 yet the distribution was sporadic and likely insufficient during

flowering and pea pod development (Table 4). Grabowska and Banaszkiewicz [22] reported a pea yield of 4.4 t ha⁻¹, which was strongly influenced by the weather conditions in central Poland during the vegetative phases, in particular at the start of flowering, and throughout the flowering stage where the precipitation deficit and high temperatures decreased yields by 87%. In our experiment, the mean temperature in May and June was also higher in 2013 than in 2014. Bueckert et al. [23] and Gantner et al. [24] have reported the strong impact of precipitation and temperature on pea yield.

Soil Tillage System	Seeds	Post-Harvest Residues	Total Biomass			
Conventional	5.19 ^a	4.41 ^a	9.59 ^a			
Reduced	4.34 ^b	3.53 ^{ab}	7.87 ^b			
Direct sowing	4.17 ^b	3.35 ^b	7.52 ^b			
SD	0.3399	0.3938	0.7009			
Year						
2013	4.29 ^A	3.46 ^A	7.76 ^A			
2014	4.83 ^A	4.06 ^A	8.90 ^A			
SD	0.2009	0.2741	0.4713			
Mean	4.56	3.76	8.33			

Table 4. Pea biomass yield (t ha^{-1}).

Values followed by the same letters in the column are not significantly different ($p \le 0.05$), SD—standard deviation.

In our experiment, the year did not significantly affect the yield of post-harvest residues (mean value $3.76 \text{ t h}a^{-1}$) and the total biomass yield ($8.33 \text{ t h}a^{-1}$), although both analyzed yields were higher in 2014 than in 2013 (Table 4). The highest yield was observed in conventional tillage ($4.41 \text{ t h}a^{-1}$) and was significantly lower (by 24%) in direct sowing. Total biomass yield was also greatest in conventional tillage ($9.59 \text{ t h}a^{-1}$) and lower in reduced tillage and direct sowing, by 18% and 21.6%, respectively.

Harvest index (HI) is an important metric in legume cultivation, calculated as $HI = (yield of seeds/yield of total biomass) \times 100$. This value highlights the proportion of seeds to post-harvest residues. The average HI value in our study over these two years was 54.7%, and was 54.1%, 55.1%, 55.4% for the conventional and reduced soil tillage systems and direct sowing, respectively. This is important when legumes are cultivated for seeds as the final product. Moreover, legumes with high HI values usually have less influence on the content of N in the soil, as it is removed from the field with the seeds.

Average spring barley yield, cultivated as a reference plant simultaneously with pea, was 6.50, 10.43 and 16.93 ha⁻¹ for the grain, post-harvest residues and total biomass, respectively (Table 5). The year resulted in significant differences between the analyzed spring barley features, with values much higher in 2014 than in 2013. The soil tillage system also resulted in significant differences in the studied features: the highest grain yield was recorded in conventional tillage (6.68 t ha^{-1}) and it was significantly lower (by 6.4%) in reduced tillage, but the difference between conventional tillage and direct sowing (6.57 t ha^{-1}) was not significant. For total biomass, the greatest yield was observed in direct sowing and was significantly lower in reduced tillage, because of the lower yield of post-harvest residues in this system. However, our results contrast with those reported by Małecka et al. [25], where the highest yields of spring barley grain, straw and total biomass were found in the reduced soil tillage system and the lowest in the direct sowing. In our study, the harvest index (HI) for barley on average for years was 38.3%. For soil tillage systems the values were as follows: 39.4% in conventional tillage, 39.4% in reduce system and 36.5% in direct sowing. The average standard deviation calculated for post-harvest residues of pea for soil tillage systems was lower than the barley plants, but on average for the years it was higher in pea plants.

Soil Tillage System	Grain	Post-Harvest Residues	Total Biomass				
Conventional	6.68 ^a	10.43 ^{ab}	16.93 ^{ab}				
Reduced	6.25 ^b	9.60 ^b	15.85 ^b				
Direct sowing	6.57 ^{ab}	11.43 ^a	18.00 ^a				
SD		0.5806	0.6729				
	Year						
2013	5.52 ^B	8.65 ^B	14.17 ^B				
2014	7.47 ^A	12.22 ^A	19.69 ^A				
SD	0.1161	0.0719	0.1476				
Mean	6.50	10.43	16.93				

Table 5. Spring barley biomass yield (t ha⁻¹).

Values followed by the same letters in the column are not significantly different ($p \le 0.05$), SD—standard deviation.

The content of N in the pea seeds and post-harvest residues was significantly different between years; higher in 2013 than in 2014 (Table 6), which was the opposite relationship to seed yield (Table 3). The seeds harvested under the different soil tillage systems conditions contained almost the same N content (mean value 3.15%). Multiplication of this value by 6.25 [26] is equal to the protein content in the seeds (19.7%). Similar protein content in pea seeds has been reported by other authors [8,27].

Table 6. The content and amount of nitrogen (N) in the pea crop.

Soil Tillage System	Total Nitrogen Content in Seeds (%)	Total Nitrogen Content in Post-Harvest Residues (%)	Total Nitrogen in Seeds (kg ha ⁻¹)	Total Nitrogen in Post-Harvest Residues (kg ha ⁻¹)	Total Nitrogen in Total Biomass (kg ha ⁻¹)
Conventional	3.18 a	0.87 a	164 a	37 a	202 a
Reduced	3.17 a	0.85 a	137 ab	30 ab	167 ab
Direct sowing	3.12 a	0.79 b	129 b	26 b	155 b
SD	0.0561	0.0109	6.6833	2.4833	9.1196
		Y	ear		
2013	3.21 A	0.86 A	138 A	29 A	167 A
2014	3.10 B	0.82 B	149 A	33 A	182 A
SD	0.0099	0.0042	5.4569	2.0276	7.4461
Mean	3.15	0.84	143	31	174

Values followed by the same letters in the column are not significantly different ($p \le 0.05$), SD—standard deviation.

The post-harvest residues contained 3.75-fold less N (mean value 0.84%) than the seeds. On average, direct sowing decreased the N content in the post-harvest residues by 0.08% compared to conventional tillage. There was no significant difference between conventional and reduced soil tillage systems.

The amount of total N harvested in the seeds, post-harvest residues and total biomass was slightly higher in 2014 than 2013, but there was no significant difference. The highest amount of N (164 kg ha⁻¹) was noted in the seeds harvested in the conventional soil tillage system and was significantly lower (by 21%) in the direct sowing. As such, total protein yield was 1025 and 806 kg ha⁻¹ in the conventional tillage and direct sowing, respectively. In their study, Małecka et al. [8] reported a lower yield of protein, although the highest protein yield was recorded with the reduced soil tillage system.

The amount of total N in the post-harvest residues was 4.6-fold less than in the seeds. If we assume that the total amount of N in the total pea biomass was 174 kg ha^{-1} (mean value), then this would suggest that 82.2% of the N was accumulated in seeds and 17.8% in the post-harvest residues (Table 6).

From an agricultural point of view, the amount of N introduced into the soil is very important in crop rotations, as it has an influence on the amount of N applied as a mineral fertilizer. The amount of N in the post-harvest residues differed between the applied soil tillage systems; the highest value (37 kg ha⁻¹) was found in conventional tillage and was significantly lower (by 30%) in direct sowing. The highest amount of total N in the total pea biomass was noted in conventional tillage (202 kg ha⁻¹) and was lower (by 47 kg ha⁻¹) in direct sowing. There was no significant difference between conventional and reduced tillage.

The value of the N harvest index, calculated as NHI = (total N in seeds/total N in biomass) \times 100, was 81.1%, 82.0% and 83.2% for the conventional tillage, reduced system and direct sowing, respectively. Lecoeur and Sinclair [28] revealed that changes in linearity of NHI dominate the N uptake by plant over seed N accumulation with the transfer of N from vegetative tissue to the seed during the early stages of seed growth. The analysis of our results also highlighted the importance of N accumulation in grain yield of field pea with average value of NHI 82.1%.

The influence of soil tillage system on N content (%) in the spring barley biomass (grain, post-harvest residues, total biomass) differed between the two years of the experiment (Table 7). Significantly higher values were noted in 2013. Soil tillage system influenced the analyzed features differently. The highest N content (%) and the amount of harvested N were always found in the conventional soil tillage system. The standard deviations calculated for total nitrogen in grain and biomass were lower for pea crops than in barley, which may indicate more stable values.

Table 7. The content and amount of nitrogen (N) in the spring barley crop.

Soil Tillage System	Total Nitrogen Content in Grain (%)	Total Nitrogen Content in Post-Harvest Residues (%)	Total Nitrogen in Grain (kg ha ⁻¹)	Total Nitrogen in Post-Harvest Residues (kg ha ⁻¹)	Total Nitrogen in Total Biomass (kg ha ⁻¹)
Conventional	1.65 ^a	0.73 ^a	109 ^a	74 ^a	183 ^a
Reduced	1.59 ^{ab}	0.65 ^b	98 ^a	62 ^b	160 ^a
Direct sowing	1.52 ^b	0.60 ^c	99 a	67 ^{ab}	166 ^a
SD	0.0378	0.0181	8.5538	2.6771	11.2250
		Ŷ	<i>'</i> ear		
2013	1.63 ^A	0.70 ^A	89 ^B	60 ^B	149 ^B
2014	1.55 ^B	0.63 ^B	115 ^A	76 ^A	191 ^A
SD	0.0153	0.0027	6.9841	2.1858	9.1652
Mean	1.59	0.66	102	68	170

Values followed by the same letters in the column are not significantly different ($p \le 0.05$), SD—standard deviation.

For the determination of biological N reduction, the most commonly used method is the isotope ¹⁵N dilution method, in which ¹⁵N is applied in the mineral N fertilizer. In this study, the pea and spring barley plants take up the isotope and the amounts that accumulate in the biomass of both crops are determined. The ¹⁵N content in the pea seeds and post-harvest residues was 2.605 and 2.987 at%¹⁵Nexcess, respectively. In both cases, the investigated factor did not have a significant influence on the value of this parameter (Table 8).

Soil Tillage System	Pea Seeds	Pea Post-Harvest Residues	Spring Barley Grain	Spring Barley Post-Harvest Residues
Conventional	2.909 ^a	3.093 ^a	3.554 ^b	3.883 ^a
Reduced	2.472 ^a	2.854 ^a	4.018 ^b	3.980 ^a
Direct sowing	2.435 ^a	3.015 ^a	4.919 ^a	4.501 ^a
SD	0.1938	0.1573	0.3896	0.2711
		Year		
2013	2.634 ^A	3.027 ^A	4.526 ^A	3.981 ^B
2014	2.576 ^A	2.948 ^A	3.801 ^B	4.262 ^A
SD	0.1048	0.1102	0.1546	0.0385
Mean	2.605	2.987	4.163	4.121

Table 8. At%¹⁵Nexcess in pea and spring barley crops.

Values followed by the same letters in the column are not significantly different ($p \le 0.05$), SD—standard deviation.

The ¹⁵N isotope content in the spring barley grain was greater in 2013 than in 2014 and was influenced by the soil tillage system (Table 8); a higher value was found in direct sowing (4.919 at%¹⁵Nexcess) and was significantly lower in the conventional and reduced soil tillage systems, by 1.365 and 0.901 at%¹⁵Nexcess, respectively. The ¹⁵N isotope content in the spring barley post-harvest residues was also significantly different between years; a higher value was found in 2014 than in 2013. In general, the ¹⁵N content in the pea biomass (seeds: 2.605 at%¹⁵Nexcess; post-harvest residues: 2.987 at%¹⁵Nexcess) was lower than in the spring barley biomass (grain: 4.163 at%¹⁵Nexcess; post-harvest residues: 4.121 at%¹⁵Nexcess). This was caused by the dilution effect: ¹⁵N taken up from the fertilizer versus atmospheric N fixed by the legumes. This has also been reported by other studies that have investigated legumes, such as white lupin [17] and faba bean [29,30].

On the basis of data contained in Table 8, the proportion of N accumulated by the pea crop from the atmosphere, fertilizer, and soil was calculated and is shown in Table 9.

Table 9. Nitrogen (N) accumulation (%) in the pea crop from different sources. Ndfa denotes the %N derived from the atmosphere, Ndff denotes the %N derived from the fertilizer and Ndfs denotes the %N derived from the soil.

Sail Tillage System	Seeds			Post-Harvest Residues			
Son mage System -	Ndfa	Ndff	Ndfs	Ndfa	Ndff	Ndfs	
Conventional	17.7 ^b	7.7 ^a	74.6 ^a	20.2 ^a	10.3 ^a	69.4 ^a	
Reduced	37.9 ^a	6.5 ^a	55.5 ^b	27.7 ^a	10.6 ^a	61.7 ^a	
Direct sowing	50.2 ^a	6.4 ^a	43.3 ^c	32.6 ^a	11.9 ^a	55.4 ^a	
SD	3.7432	1.3348	2.4197	7.7257	1.0058	8.6869	
Year							
2013	40.5 ^A	6.9 ^A	52.6 ^B	23.7 ^A	10.6 ^A	65.6 ^A	
2014	30.0 ^A	6.8 ^A	63.2 ^A	29.9 ^A	11.3 ^A	58.7 ^A	
SD	3.0563	1.0899	1.9757	6.3080	0.8212	7.0928	
Mean	35.2	6.8	57.9	26.8	10.9	62.1	

Values followed by the same letters in the column are not significantly different ($p \le 0.05$), SD—standard deviation.

The N accumulated in the seeds (expressed as the mean value from both years of the experiment and across all soil tillage systems) was as follows: 35.2% from the atmosphere, 6.8% from the fertilizer and 57.9% from the soil. The differences were strongly influenced by the soil tillage system with the highest percentage found in direct sowing (50.2%) and was significantly lower (by 32.5%) in the conventional soil tillage system, the same results were found in research of Reiter et al. [31]. Difference in the % Ndfa between the soil tillage systems is likely caused by the mineral forms of nitrogen in soil. The conventional soil

tillage system introduces substantial oxygen into the soil, which accelerates the mineralization of soil organic N compounds, releasing mineral forms of N that inhibit the infection process of *Rhizobium*, as well as the biological N reduction process [32]. Mineral forms of N inhibit the nodulation process and decrease the activity of nitrogenize [33–36].

There were no differences in the N content in the pea seeds and the post-harvest residues derived from the applied mineral fertilizer (Table 9). A greater percentage of N was derived by the pea crop from the soil than the atmosphere. The highest amount was taken from the soil in conventional tillage (74.6%) and was significantly lower in the reduced system (by 19.1%) and direct sowing (by 31.3%).

In the post-harvested residues, the percentage of N derived from the atmosphere was 26.8% and there was no significant difference between years and tillage system.

The amount of N in the plant derived from the fertilizer was much lower than that derived from the atmosphere and the soil (mean value for whole experiment: 10.9%), and no differences between the year of experiment and the tillage system were observed. The majority of nitrogen content in the pea post-harvest residues was derived from the soil (62.1%); slightly higher in 2013 than in 2014. In addition, the soil tillage systems resulted in slightly different amounts of N derived from the soil: highest in conventional tillage (69.4%) and lowest (55.4%) in direct sowing.

Lower standard deviations values obtained for discussed features in pea may suggest more stable values, in comparison to barley crop.

For legume cultivation, it is crucial to define how much N is fixed from the atmosphere. In our case, N fixed from the atmosphere in total biomass was 56.5 kg ha⁻¹, with 48.4 kg ha⁻¹ accumulated in the harvested seeds (Table 10). The highest amount (64.6 kg ha⁻¹) occurred in direct sowing and was significantly lower (55.1%) in the conventional soil tillage system. Although, the rainfall at the turn of May and June during the flowering of pea in 2013 was higher than in 2014, the temperature was lower (Table 1). This situation could decrease the nitrogen fixation from the atmosphere in 2013.

Table 10. Nitrogen (N) accumulation (kg ha⁻¹) by the pea crop from different sources. Ndfa denotes the N derived from the atmosphere, Ndff denotes the N derived from the fertilizer and Ndfs denotes the N derived from the soil.

Soil Tillage System	Seeds			Post-Harvest Residues		
John Thiage System –	Ndfa	Ndff	Ndfs	Ndfa	Ndff	Ndfs
Conventional	28.9 ^b	12.5 ^a	123.0 ^a	7.4 ^a	3.8 ^a	26.1 ^a
Reduced	52.0 ^a	8.9 ^a	76.4 ^b	8.3 ^a	3.1 ^b	18.5 ^a
Direct sowing	64.4 ^a	8.3 ^a	57.0 ^c	8.5 ^a	3.1 ^b	14.3 ^a
SD	4.4402	1.8042	2.9402	2.0692	0.1472	4.4364
		Ye	ar			
2013	53.8 ^A	9.7 ^A	74.6 ^B	6.8 ^A	3.0 ^B	19.3 ^A
2014	43.0 ^A	10.1 ^A	96.3 ^A	9.3 ^A	3.6 ^A	19.9 ^A
SD	3.6254	1.4731	2.4007	1.8667	0.1202	3.6223
Mean	48.4	9.9	85.4	8.0	3.2	19.6

Values followed by the same letters in the column are not significantly different ($p \le 0.05$), SD—standard deviation.

In the post-harvest residues, the amount of N fixed from the atmosphere was very low (only 8.0 kg ha⁻¹) and was not significantly different between soil tillage systems. Carrance et al. [37] reported that about 50% of the N contained in pea seeds is derived from the atmosphere and about 20 kg ha⁻¹ of the N in straw. Enrico et al. [38] reported that 60% of N demand by field peas in Argentina (average from 16 experiments) was met by biologically fixated N.

The ¹⁵N isotope method allowed the amount of N in the pea biomass derived from the three sources to be determined. It was also possible to determine the ¹⁵N accumulation in each part of the pea biomass. The amount of ¹⁵N that accumulated in the seeds was

not different between years but only by soil tillage system (Table 11). The highest amount (4.74 kg ha⁻¹) was recorded in the conventional tillage and was significantly lower in the reduced system (28.3%) and direct sowing (33.1%). Mean total ¹⁵N accumulated in the pea biomass was 4.69 kg ha⁻¹, with the highest value observed in the conventional system (5.89 kg ha⁻¹), and less in the reduced tillage (4.25 kg ha⁻¹) and direct sowing (3.95 kg ha⁻¹). The total N accumulated in the pea biomass in the conventional tillage we estimated as 52.8%, 38.1% in the reduced system, and 35.4% in the direct sowing (overall mean value: 42.1%).

Table 11. The accumulation of 15 N isotope in the pea biomass in relation to the amount applied as $({}^{15}NH_4)_2SO_4$.

Specification	So	Soil Tillage System						
operation	Conventional	Reduced	Direct Sowing	2013	2014			
Quantity of 15 N in seeds (kg ha ⁻¹)								
Mean	4.74 ^a	3.40 ^b	3.17 ^b	3.70 ^A	3.84 ^A			
SD		0.1266		0.0	618			
% of ¹⁵ N applied	42.5	30.5	28.4	33.	8 *			
Quantity of 15 N in post-harvest residues (kg ha ⁻¹)								
Mean	1.15 ^a	0.85 ^{ab}	0.78 ^b	0.88 ^B	0.97 ^A			
SD		0.0211		0.0	023			
% of ¹⁵ N applied	10.3	7.6	7.0	8.	3 *			
In total biomass (kg ha ^{-1})	5.89	4.25	3.95	4.6	9 *			
Accumulation of applied ¹⁵ N (%)	52.8	38.1	35.4	42.	1*			
	Accumulation of ¹⁵ N (%)							
Seeds	80.5	80.0	80.3	80.	3 *			
Post-harvest residues	19.5	20.0	19.7	19.	7*			
Total	100	100	100	10	0 *			

* Mean; Values followed by the same letters in the column are not significantly different ($p \le 0.05$), SD—standard deviation.

Similar results have been reported in a white lupin experiment [17]. Studies have shown that more than 50% of the applied ¹⁵N isotope in mineral fertilizer (¹⁵NH₄)₂SO₄ may remain in the soil as organic and mineral compounds of N, be lost by deep infiltration with water or by gases (denitrification process) or be incorporated into the root system of the pea plant [4,39]. If we set the total amount of ¹⁵N isotope by the pea crop at 100%, then we estimate that 80.3% accumulated in the seeds and 19.7% in the post-harvest residues. These data confirm that legumes accumulate N mainly in their seeds, which is important information for food production, although from a soil fertility perspective, less N is introduced into the soil to provide a future source of N for subsequent plants in the crop rotation.

The practically functional relations were found between the seed yield and the total biomass in each soil tillage system in pea and barley plants. But in reduced tillage systems, correlations between the total nitrogen content in seeds (%) and seed yield were also strong relations. Such strong functional relations were also found in barley plants in the reduced system between total nitrogen content in post-harvest residues (%) and grain yield and total biomass yield (Figure 1).



 $0 \le r < 0.2$ – practically no relation between characters;

 $0.2 \le r < 0.5$ – poor relation between characters;

 $0.5 \le r < 0.75 - medium relation;$

 $0.75 \le r < 0.95 - \text{strong relation};$

 $0.95 \le r < 1.00$ – practically functional relation.

Figure 1. Correlation coefficients between features analyzed for pea (**P**) and barley (**B**) in soil tillage systems. Features compared: Y, seed/grain yield; TY, total biomass yield; TNS, total nitrogen content in seeds (%); TNPH, total nitrogen content in post-harvest residues (%). Interpretation of Pearson's linear correlation coefficient: $0 \le p < 0.2$ —practically no relation between characters; $0.2 \le p < 0.5$ —poor relation between characters; $0.5 \le p < 0.75$ —medium relation; $0.75 \le p < 0.95$ —strong relation; $0.95 \le p < 1.00$ —practically functional relation.

In a study carried out by Panasiewicz et al. [40], the protein content ranged from 295 g kg⁻¹ in conventional tillage to 314 g kg⁻¹ in no-tillage (direct sowing) and the tillage system did not influence the protein content in narrow-leaved lupin seeds. However, there was a trend of increasing protein content in seeds as the tillage system became more minimized. The Pearson's linear correlation showed a practical functional relationship between seed yield and protein efficiency.

4. Conclusions

The soil tillage system was found to significantly influence the yield in all parts of the pea plant. The greatest yield was observed in conventional tillage and was significantly lower in direct sowing. The percentage of N derived from the atmosphere was significantly higher in direct sowing compared to conventional tillage. The N that accumulated in the pea biomass was derived from three sources: atmosphere (35.2%), mineral N fertilizer (6.8%) and the soil (57.9%). The modified percentage of N derived from the atmosphere was significantly influenced by the soil tillage system; the lowest value (17.7%) was observed

in conventional tillage and the highest in direct sowing (50.2%), what equates to 28.9 and 64.4 kg ha⁻¹, respectively. The amount of ¹⁵N applied in the mineral fertilizer (¹⁵NH₄)₂SO₄ was 11.14 kg ha⁻¹, a portion of which accumulated in the pea biomass (4.69 kg), which equates to 42.1% recovery. If we set the total amount of ¹⁵N isotope by the pea crop at 100%, then we estimate that 80.3% accumulated in the seeds and 19.7% in the post-harvest residues.

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