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Effects of the Preceding Crop on Soil N Availability, Biological Nitrogen Fixation, and Fresh Pod Yield of Organically Grown Faba Bean (*Vicia faba* L.)

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Abstract: In the current study, the impact of the preceding crops on growth, fresh pod yield, nitrogen fixation efficiency, and nitrogen nutrition of faba bean (*Vicia faba* L.) was investigated for two years in both organic and conventional crops. As preceding crops served cabbage, pea, and faba bean. The pod number per plant (PN) and the total fresh pod yield (TFPY) were significantly lower with cabbage compared to pea and faba bean as preceding crops in both cropping systems and both experimental years. However, in the organic farming system, pea increased significantly in PN and TFPY compared to faba bean as a preceding crop, while in the conventional system, there was no significant difference between the two legumes. The greater yield performance with the two legumes as preceding crops was associated with higher soil NO_3-N and total-N concentrations at the beginning of the subsequent faba bean crop. The higher soil N availability when the preceding crop was a legume resulted partly from the higher biomass of crop residues left by these crops on the field after harvest, compared to cabbage. However, it was also associated with a more extensive nodulation of the faba bean roots by rhizobia and a higher percentage of N derived from atmosphere (%Ndfa) in their plant tissues, as determined through the natural abundance of the 15N isotope, when the preceding crop was a legume. The cropping system had no impact on pod yield, but organic farming increased the %Ndfa in both years.

Keywords: biological nitrogen fixation; cabbage; conventional; faba bean; legume; organic; pea; nodulation; soil nitrogen

1. Introduction

Faba bean (*Vicia faba* L.) is an important legume crop globally, and it is utilised both as a pulse and a vegetable crop for fresh pod consumption. The dry and fresh seeds or pods are recommended for their benefits to human nutrition as a dietary source of fibre and protein [1]. Furthermore, the dry seeds of faba bean are also important in animal nutrition, mainly because of their high protein content, which can be higher than 30% on a dry matter basis, but also due to the energy supply, as its starch content usually exceeds 40% [2]. Furthermore, faba bean can be used as green manure before establishing the next crop, contributing to a substantial reduction or even elimination of synthetic fertiliser application. The application of fresh faba bean biomass as mobile green manure, significantly increased the available plant N in the soil and, concomitantly, the plant N nutrition and the fruit yield in organic tomato crops that were cultivated in greenhouses [3]. Crop productivity of faba bean is typically affected by various soil–plant interactions that may influence soil fertility, root system development, and crop photosynthesis. However, little is known about the factors that can have an impact on faba bean production and biological N_2 fixation when this legume species is integrated in crop rotation systems [4].

Usually, more than 95% of N in surface soils is present in organic forms. The remainder is in mineral forms, including some fixed NH_4^+ [5]. The organic N in the soil can be divided into two broad categories: (i) organic residues, undeclared plant and animal residues and partially decomposed products, and (ii) soil organic matter or humus. Organic N plays a crucial role in plant nutrition through direct and indirect effects on microbial activity and nutrient availability [6].

The ability of legumes to fix atmospheric N_2 and add external N to the plant–soil ecosystem is a distinct benefit provided by this botanical family. The quantities of biologically fixed N by legumes per year vary significantly from zero to several hundred kg N ha^{-1}. For instance, the quantities of symbiotically fixed N_2 by faba bean under field conditions that have been reported by several investigators, range from 15 [7] to 648 kg N ha^{-1} [8]. This wide range results mainly from a high variation in specific growing conditions, genotypic variations, and methods used to quantify biological nitrogen fixation [9]. Variables affecting the quantity of fixed atmospheric N_2 include legume species and cultivar, soil type and texture, pH, soil NO_3-N level, soil temperature and water regimes, availability of other nutrients, and crop and harvest management [10]. In experiments performed in Canada, decomposition of faba bean applied as green manure or as crop residues considerably increased the soil microbial population [11].

Organic farming represents a promising approach to reconcile food production with environmental protection and multiple ecosystem service deliveries [12–14]. It is an alternative production concept that promotes crop diversification. Synthetic fertilisers and pesticides are banned in organic farming, therefore, crop rotations are supposed to have a strategic role in this farming system. Their aim is to control endemic plant diseases, maintain soil fertility, increase the input of symbiotically fixed N_2 to the agroecosystem, and reduce agrochemical applications without restricting crop production. Diversified crop rotations are adopted in organic systems to sustain crop yields by providing alternative levers for pest control and nutrient management [14]. From an agronomical point of view, including faba bean in crop rotation systems improves soil N via biological nitrogen fixation process and increases soil organic matter. Hence, the inclusion of faba bean in rotation systems greatly contributes to improvements in the sustainability of agricultural systems [1].

In rotations, the selection and sequence of crops are determined by a combination of agronomic and economic factors and principles and standards of organic farming [15]. Even though crop rotations have been dramatically simplified over the last 50 years, our understanding of their impact on productivity is surprisingly limited [14]. As a result, the magnitude and variability of yields in rotated crops and their interactions with field management practices and environmental factors when implementing crop rotations remain uncertain [16].

Understanding the effects of the preceding crop on the following crop is of fundamental importance for the establishment of effective crop rotations. Considering this background, this study was commissioned to investigate the responses of faba bean to the placement of a legume (pea) or a non-legume (cabbage) plant as a preceding crop in a rotation system. To better understand the mechanisms underlying the recorded responses, the cultivation of faba bean after faba bean was also tested as a control, while all crop sequencing treatments were applied in both an organic and a conventional farming system.

2. Materials and Methods

2.1. Site Description

Two field experiments with faba bean (*Vicia faba* L.) as a cropping plant and three different preceding crops were conducted in a field certified for organic cultivation at the experimental farm of the Agricultural University of Athens in Kopais, located in central Greece (23°05′41′′ E, 38°23′51′′ N, altitude 95 m). The certification took five years, according to the legislation governing organic farming. The first experiment was carried out from

November 2015 to June 2016, and the second experiment from November 2016 to June 2017. The mean air temperature and precipitation in the experimental site during the two growing seasons are presented in Figure 1.

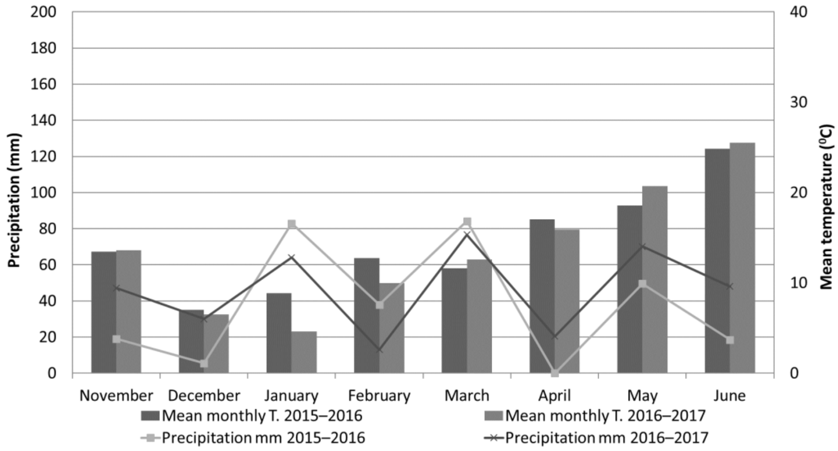

Figure 1. Mean monthly temperatures and monthly precipitation during the two experimental years (first EY, November 2015–June 2016; second EY, November 2016–June 2017).

At the beginning of each experiment, soil samples were collected from the plough layer (0–30 cm) to determine their physical and chemical properties. The soil texture, which was determined by applying the Bouyoucos hydrometer method [17], was 43.7% clay, 25.6% silt, and 30.7% sand. The percentage of organic matter, which was analysed according to the method of Nelson and Sommers [18], was 10.83%. The soil pH and electrical conductivity were 8.12 and 0.75 mS cm^{-1}, respectively, as determined in a soil to water suspension of 1:2 v/v using conductivity and pH meters. The total-N, P and K concentrations in the soil, which were determined as described in a previous paper [19], were 0.27% (*w*/*w*), 28, and 137 mg kg^{-1}, respectively.

2.2. Experimental Layout

The two rotation experiments were laid out as split-plot designs with four replications and two experimental factors. The farming system (conventional or organic) was the main plot, while the preceding cropping was the sub-plot within each main plot. After a pre-evaluation of faba bean and pea varieties and landraces [20,21], the faba bean landrace' AUALEFKADAfb001', the pea landrace 'AUAANDRO001', and the commercial cabbage hybrid 'Krautkaiser F1' were selected for testing in both experiments. The preceding crops for faba bean (F) were cabbage (C), pea (P), and faba bean, corresponding to the treatments (C-F), (P-F), and (F-F), respectively. The plots with the different treatments were randomised within the main plot. Each sub-plot size was 10.5 m^2 (3 × 3.5 m) and comprised 10 rows with plants spaced at 30 cm apart. Faba bean seeds in the two experimental years were sown by hand to a depth of 2–3 cm on 12 November 2015 and 22 November 2016, respectively. Following local cropping practices, which were validated in a previous study (20), the quantities of fertilisers applied before sowing in both EYs were 714 kg ha^{-1} of an inorganic NPK fertiliser (N:P_2O_5:K_2O ratio 11:15:15) in the conventional plots and 7.15 t ha^{-1} sheep manure in the organic plots. The corresponding N application rates were 78.55 and 60.05 kg ha^{-1}, respectively. The rate of sheep manure application is given on a dry weight (DW) basis. The concentrations of primary nutrients in the sheep manure were as follows: 0.84% total-N, 0.3% P_2O_5, 0.7% K_2O_5, 0.38% CaO, and 0.24% MgO, on a DW basis. In the second experiment, some more fertilisers were applied in addition to the base dressing during the crop season. More specifically, 161.9 kg ha^{-1} of patentkali (K_2O:MgO:SO_3 ratio 30:10:42.5) was applied on both farming systems and 23.8 kg ha^{-1} of ammonium nitrate fertiliser (17.5% NH_4-N and 17.5% NO_3-N) was applied only in the conventional farming system on 23 March. In both years, no herbicide was applied; weeds were controlled manually in both farming systems. In both experimental years, there were no serious pest and disease infections, and, thus, no pesticides or insecticides were applied to the crops.

2.3. Fresh Pod Yield and Yield Components

When faba bean reached the stage of commercial maturity for fresh pod production, all pods from 10 plants were hand-harvested at three successive dates in each sub-plot avoiding border plants. Number, length, and total weight of fresh pods per cultivated area unit were recorded separately in each plot.

2.4. Soil and Plant Tissue Analysis

Soil samples were collected at three developmental stages of faba bean crop: before crop establishment (BCE), at the flowering stage (FS), i.e., when more than 50% of plants achieved this stage, and at the final harvest stage (FHS). A cylindrical auger with a diameter of 10 cm and a height of 20 cm was used. For chemical analyses, each soil sample was air-dried and sieved to <2 mm. For the determination of the NO_3-N concentrations in the soil samples, the methodology described by Ntatsi et al. [20] was followed and the measurements were conducted using a microplate spectrophotometer (Anthos Zenyth 200; Biochrom, Holliston, MA, USA). To determine soil K, soil samples were extracted with ammonium acetate solution 1 N and pH 7. Then soil samples were shaken mechanically for 5 min and remained at rest until the supernatant liquid became clear. The filtration was performed with Whatman No 42 filters (ash less). The soil K concentration was determined in this extract by flame photometry (Sherwood Model 410, Cambridge, UK). Soil subsamples from samples collected at the BCE and FHS stages were further used to determine the soil total-N content (%). Briefly, the samples were air-dried, ground with a suitable laboratory mill, sieved to <0.14 mm and subsequently used for total-N analysis by applying the Kjeldahl method.

2.5. Root Nodulation

Two root samples of faba bean plants from each sub-plot were collected to determine root nodulation. For root samples, a cylindrical auger with a diameter of 10 cm and a height of 20 cm was used. Samples were placed for 24 h in a Calgon solution (dispersing agent), prepared by adding 40 g $(NaPO_3)_6$ and 10 g Na_2CO_3 per 1000 mL of water. Subsequently, the roots were separated from the soil by soaking in water and then gently washing over a series of sieves with mesh sizes of 2.0 and 0.5 mm, as described by Anderson and Ingram [22]. Afterwards, the number of nodules per L soil was counted.

2.6. Plant Tissue Analysis and Biological Nitrogen Fixation

Biological nitrogen fixation was estimated using the natural ^{15}N abundance method. First, the ^{15}N content of the plant samples was determined at the Stable Isotope Facility of UC-Davis, CA, USA, by CF-IRMS (Europa Scientific, Crewe, UK). This was used to compute the differences ($\delta^{15}N$) between ^{15}N of sample and natural ^{15}N abundance in the atmospheric N, which is a known constant (0.3663%). Then, the $\delta^{15}N$ values were estimated as parts per thousand (‰) deviations relative to the nominated international standard of atmospheric N_2 (0.3663%) using the following equation [23]:

$$\delta^{15}N\ (‰) = \left(\frac{atom\%^{15}Nsample - 0.3663}{0.3663}\right) * 1000 \tag{1}$$

The proportion of N derived from the atmosphere (%*Ndfa*) was estimated by substituting the $\delta^{15}N$ (‰) of the N_2-fixing legume and a non-N_2-fixing reference plant grown on the same soil by employing the following equation referenced by Unkovich et al. [24]:

$$\%Ndfa = \left(\frac{\delta^{15}N\ of\ reference\ plant - \delta^{15}N\ of\ legume}{\delta^{15}N\ of\ reference\ plant - B}\right) * 100 \tag{2}$$

where 'B' is the $\delta^{15}N$ (‰) of faba bean shoots grown on an inert medium and fully starved of N throughout their life, thereby being entirely dependent upon N_2 fixation. The B value used in the current study was −0.5, as suggested by Unkovich et al. [24]. The reference plant used in the current study was head cabbage (*Brassica oleracea* var. capitata). Reference plants were separately collected from organic and conventional plots and used to determine the corresponding $\delta^{15}N$ values.

The total amount of biologically-fixed N_2 contained in the aboveground dry biomass (DB) of faba bean (BNF, kg ha^{-1}) was calculated using the following equation [25]:

$$BNF = \frac{DB * N * Ndfa}{100} \quad (3)$$

where *DB* is the dry biomass in kg ha^{-1}, *N* is the total N concentration (%) in the aboveground dry biomass, and *Ndfa* is the percentage of N derived from the atmosphere with reference the total N content of the epigeous dry biomass.

Plant samples collected at the FS stage were used to determine the total-N and K concentrations in the dried shoot of faba bean. The entire shoot of each plant sample was dried in a forced-air oven at 65 °C to constant weight and homogenised. Subsequently, a subsample was ground to powder. The total N concentration was determined in a subsample of the ground shoot by applying a direct distillation method based on steam distillation (KjeltecTM 8200 Distillation Unit, FOSS Analytical A/S, Hillerød, Denmark). To determine the tissue K concentration, a subsample of the dried and ground shoot was subjected to dry ashing at 550 °C and extracted with 1 N HCl. The K concentration was determined in this extract by flame photometry (Sherwood Model 410, Cambridge, UK).

2.7. Statistical Analysis

A two-factorial analysis of variance (ANOVA) was applied to evaluate the main effects of the farming systems (Factor 1), the preceding crop (Factor 2), and the interactions between them. Climate data are shown in a bar graph, while all other data are presented in tables as means ± SE (n = 4). In addition, the Duncan's multiple range test was performed for all parameters measured when the ANOVA was significant at the $p \leq 0.05$ level or lower. The statistical analysis was performed using the STATISTICA software package, version 9.0 for Windows (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. Soil Mineral and Total N Concentrations

In both experimental years, the soil NO_3-N concentration was significantly higher in the conventional farming system at all crop stages, regardless of the crop sequence. However, the differences were significant only in the second EY (Table 1). The cultivation of a legume crop (pea or faba bean) before faba bean resulted in higher soil NO_3-N levels in both farming systems and experimental years, although the differences were not always significant. No interactions between the two experimental factors were found and, therefore, only the main effects are presented in Table 1. The soil NH_4-N concentrations ranged from 3.6 to 6.0 mg kg^{-1} without any significant differences or interactions between the experimental treatments and factors (data not shown).

Overall, the soil total-N was significantly higher in the organic compared to the conventional farming system, although, in the first EY, the difference was insignificant at the FHS (Table 2). In both EYs, the total-N levels were significantly higher when faba bean followed a legume crop compared to cabbage in both farming systems. In the first EY, the soil total N BCE was significantly higher when faba bean was followed by faba bean compared to pea in the organic farming system, while it was not influenced by the preceding crop in the conventional system.

Table 1. Impact of the farming system (organic or conventional) and the preceding crop (cabbage, pea, and faba bean: C, P, and F, respectively) on soil NO_3^--N concentrations (mg kg^{-1}) at three cropping stages of faba bean in both EYs.

	EY 2015–2016			EY 2016–2017		
	BCE	**FS**	**FHS**	**BCE**	**FS**	**FHS**
Main effects						
Farming system						
Organic	15.90[1] ± 0.31 [a]	16.37 ± 0.24 [a]	16.10 ± 0.03 [a]	14.04 ± 0.16 [b]	11.87 ± 0.14 [b]	13.90 ± 0.25 [b]
Conventional	16.33 ± 0.27 a	16.58 ± 0.21 [a]	16.89 ± 0.28 [a]	15.45 ± 0.15 [a]	13.21 ± 0.13 [a]	15.10 ± 0.18 [a]
Preceding crop						
C-F	14.91 ± 0.02 [b]	16.48 ± 0.01 [a]	15.20 ± 0.05 [b]	13.94 ± 0.27 [b]	12.06 ± 0.26 [a]	13.57 ± 0.13 [b]
P-F	17.14 ± 0.09 [a]	17.45 ± 0.02 [a]	17.63 ± 0.06 [a]	15.16 ± 0.21 [a]	12.92 ± 0.25 [a]	14.74 ± 0.07 [a]
F-F	16.29 ± 0.09 [a]	15.48 ± 0.02 [a]	16.65 ± 0.03 [a]	15.14 ± 0.30 [a]	12.64 ± 0.26 [a]	15.20 ± 0.07 [a]
Statistical significance						
Farming system (FS)	ns	ns	ns	***	*	*
Preceding crop (PC)	*	ns	*	**	ns	*
FS × PC	ns	ns	ns	ns	ns	ns

Values are means (n = 4) ± standard errors. In each column, means within the same factor followed by different letters indicate significant differences according to the Duncan's multiple range test: *, ** and *** indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively; ns = not significant. BCE: before crop establishment; FS: flowering stage; FHS: final harvest stage.

Table 2. Impact of the farming system (organic or conventional) and the preceding crop (cabbage, pea, and faba bean: C, P, and F, respectively) on soil total-N (%) content at two stages of faba bean in both EYs.

Farming System	Preceding Crop	Total-N (%) 2015–2016		Total-N (%) 2016–2017	
		BCE	**FHS**	**BCE**	**FHS**
Organic	C-F	0.485 ± 0.001 [c]	0.507 ± 0.001	0.522 ± 0.011 [b]	0.514 ± 0.004 [b]
	P-F	0.554 ± 0.010 [b]	0.531 ± 0.011	0.563 ± 0.014 [a]	0.543 ± 0.010 [a]
	F-F	0.592 ± 0.003 [a]	0.546 ± 0.004	0.577 ± 0.015 [a]	0.554 ± 0.006 [a]
Conventio-nal	C-F	0.490 ± 0.003 [c]	0.486 ± 0.011	0.487 ± 0.006 [c]	0.479 ± 0.011 [c]
	P-F	0.467 ± 0.001 [c]	0.519 ± 0.010	0.514 ± 0.010 [b]	0.504 ± 0.006 [b]
	F-F	0.480 ± 0.008 [c]	0.527 ± 0.008	0.509 ± 0.008 [b]	0.487 ± 0.003 [c]
Main effects					
Organic		0.544 ± 0.013	0.528 ± 0.006 [a]	0.554 ± 0.010	0.537 ± 0.006
Conventional		0.479 ± 0.004	0.511 ± 0.007 [a]	0.503 ± 0.005	0.490 ± 0.005
C-F		0.488 ± 0.002	0.497 ± 0.006 [b]	0.505 ± 0.009	0.497 ± 0.008
P-F		0.511 ± 0.016	0.525 ± 0.007 [a]	0.539 ± 0.013	0.524 ± 0.014
F-F		0.536 ± 0.021	0.537 ± 0.005 [a]	0.543 ± 0.014	0.521 ± 0.009
Statistical significance					
Farming system (FS)		**	ns	*	*
Preceding crop (PC)		*	*	**	**
FS × PC		**	ns	*	**

Values are means (n = 4) ± standard errors. For each factor, or for all factorial combinations when the interaction was significant, means within the same column followed by different letters indicate significant differences according to the Duncan's multiple range test: * and ** indicate significance at $p < 0.05$, $p < 0.01$, respectively; ns = not significant. BCE: before crop establishment; FHS: final harvest stage.

3.2. Soil K Concentrations

The soil K concentration was significantly higher in the conventional compared to the organic farming system at all three cropping stages of faba bean, in both EYs (Table 3), although BCE and at the FS in the second EY some differences were insignificant. Furthermore, the cultivation of faba bean after cabbage resulted in higher soil K concentrations compared to pea or faba bean as preceding crops although the differences were not always significant.

Table 3. Impact of the farming system (organic or conventional) and the preceding crop (cabbage, pea, and faba bean: C, P, and F, respectively) on soil K concentrations (mg kg^{-1}) at three cropping stages of faba bean in both EYs.

Farming System	Preceding Crop	EY 2015–2016			EY 2016–2017		
		BCE	FS	FHS	BCE	FS	FHS
Organic	C-F	165.00± 11.22	144.50 ± 2.50	109.50 ± 2.21	210.00 ± 7.70 [a]	212.37 ± 4.62 [a]	173.07 ± 8.98
	P-F	130.62 ± 6.87	132.00 ± 1.29	107.00 ± 1.29	140.00 ± 6.69 [b]	176.25 ± 5.86 [b]	136.13 ± 7.26
	F-F	130.63 ± 13.16	135.50 ± 1.50	107.50 ± 1.50	153.12 ± 13.16 [b]	168.38 ± 6.25 [b]	133.38 ± 7.23
Conventional	C-F	185.62 ± 6.78	158.50 ± 0.95	115.00 ± 1.29	218.13 ± 12.88 [a]	213.18 ± 5.29 [a]	189.00 ± 6.89
	P-F	178.75 ± 7.93	140.50 ± 0.95	112.50 ± 0.95	213.75 ± 10.72 [a]	205.87 ± 1.97 [a]	169.13 ± 6.87
	F-F	171.87 ± 6.87	134.00 ± 1.82	116.50 ± 1.82	218.13 ± 10.42 [a]	219.38 ± 7.59 [a]	162.25 ± 7.93
Main effects							
Organic		142.08 ± 7.44 [b]	137.33 ± 1.62 [b]	108.00 ± 0.95 [b]	167.70 ± 10.44	185.66 ± 6.47	147.52 ± 6.82 [b]
Conventional		178.75 ± 4.14 [a]	144.33 ± 1.11 [a]	114.50 ± 0.85 [a]	210.00 ± 6.49	212.81 ± 3.30	173.46 ± 5.10 [a]
	C-F	175.31 ± 7.23 [a]	146.00 ± 1.36 a	112.25 ± 1.57 [a]	204.06 ± 7.30	212.78 ± 3.25	181.03 ± 6.04 [a]
	P-F	154.68 ± 10.31 [b]	137.75 ± 1.27 [b]	109.75 ± 1.27 [a]	176.87 ± 15.11	191.06 ± 6.28	152.63 ± 7.76 [b]
	F-F	151.25 ± 10.39 [b]	139.75 ± 1.94 [b]	111.75 ± 1.94 [a]	185.62 ± 14.53	193.87 ± 10.65	147.82 ± 7.38 [b]
Statistical significance							
Farming system (FS)		***	***	***	***	***	***
Preceding crop (PC)		*	***	ns	ns	**	***
FS × PC		ns	ns	ns	**	**	ns

Values are means (n = 4) ± standard errors. For each factor, or for all factorial combinations when the interaction was significant, means within the same column followed by different letters indicate significant differences according to the Duncan's multiple range test: *, ** and *** indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively; ns = not significant. BCE: before crop establishment; FS: flowering stage; FHS: final harvest stage.

3.3. Root Nodulation

When faba bean was grown organically, the highest number of rhizobia-induced nodules in roots was recorded when the preceding crop was faba bean, followed by pea as the preceding crop, while the lowest number was recorded with cabbage as preceding crop in both EYs (Table 4). In the conventional system, the number of root nodules was similar when the preceding crop was a legume, but significantly lower when the preceding crop was cabbage.

Table 4. Impact of the farming system (organic or conventional: O or C, respectively) and the preceding crop (cabbage, pea, and faba bean: C, P, and F, respectively) on number of nodules per volume of soil (No L^{-1}) in the roots of faba bean in both EYs.

Farming System	Preceding Crop	EY 2015–2016 (No L^{-1} of Soil)	EY 2016–2017 (No L^{-1} of Soil)
Organic	C-F	75.38 ± 2.47 [b]	61.08 ± 2.40 [c]
	P-F	99.19 ± 2.93 [a]	78.79 ± 2.83 [b]
	F-F	103.25 ± 3.12 [a]	92.05 ± 2.97 [a]
Conventional	C-F	47.90 ± 2.42 [c]	39.53 ± 2.75 [d]
	P-F	68.87 ± 2.46 [b]	60.96 ± 2.67 [c]
	F-F	73.69 ± 2.81 [b]	60.07 ± 1.83 [c]

Table 4. *Cont.*

Farming System	Preceding Crop	EY 2015–2016 (No L^{-1} of Soil)	EY 2016–2017 (No L^{-1} of Soil)
Main effects			
Organic		92.60 ± 3.72	77.31 ± 3.84
Conventional		63.48 ± 3.38	59.19 ± 2.97
	C-F	61.64 ± 5.21	50.31 ± 4.06
	P-F	84.03 ± 5.82	69.88 ± 3.45
	F-F	88.47 ± 5.58	76.06 ± 6.10
Statistical significance			
Farming system (FS)		**	**
Preceding crop (PC)		***	***
FS × PC		***	***

Values are means (n = 4) ± standard errors. Means within the same column followed by different letters indicate significant differences according to the Duncan's multiple range test: ** and *** indicate significance at $p < 0.01$, $p < 0.001$, respectively; ns = not significant.

3.4. Tissue K Concentration

In the first EY, the K concentration in the dry shoot of faba bean was not influenced by the farming system or the preceding crop. (Figure 2). However, in the second EY, the shoot K concentration was significantly higher in the conventional compared to the organic farming system. The preceding crop had no significant impact on the K concentration in the shoot of faba bean.

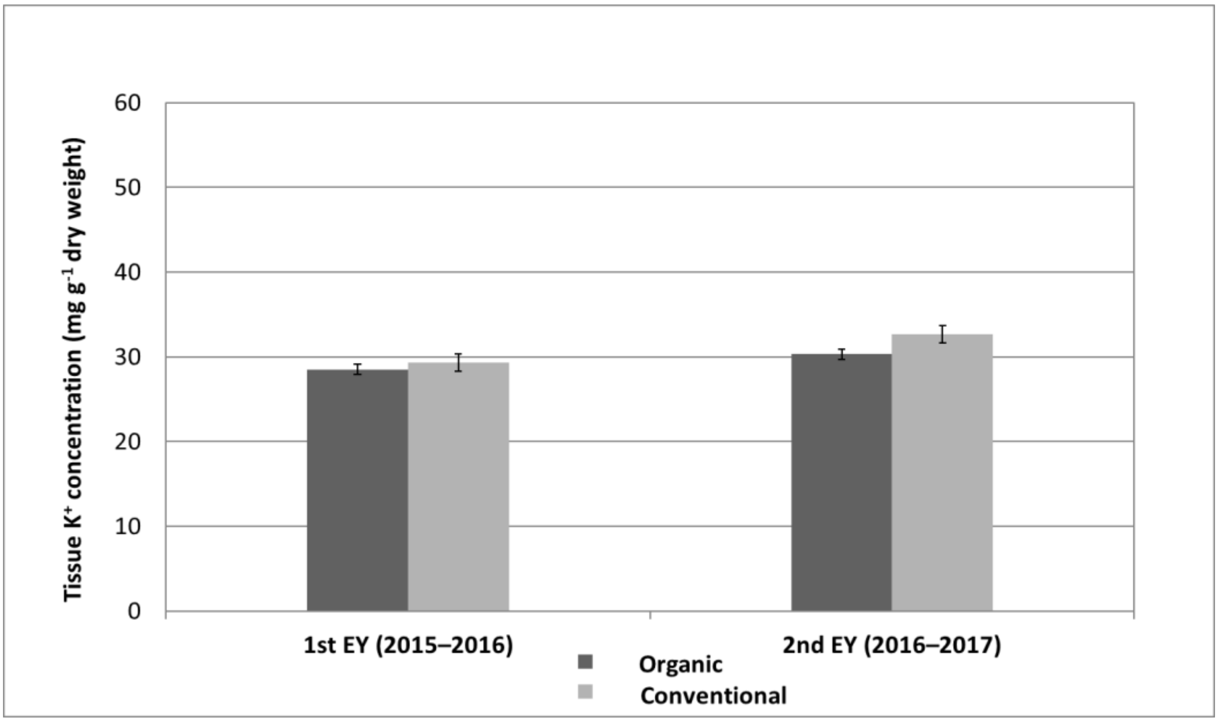

Figure 2. Impact of the farming system (organic or conventional: O or C, respectively) on K concentrations (mg g^{-1}) in the shoot of faba bean at the flowering stage in both EYs. Vertical bars denote ±standard errors of means (n = 4). For each EY, different letters above the bars denote significant differences according to the Duncan's multiple range test at $p < 0.05$.

3.5. Biological Nitrogen Fixation

The percentage of N derived from the atmosphere (%Ndfa) in the shoot biomass of faba bean was significantly higher in the organic farming system, but only when the preceding crop was a legume (pea or faba bean), while the cultivation of faba bean after cabbage resulted in similar %Ndfa in the two farming systems (Table 5). A similar trend was also observed in the second EY, but in that experiment, the difference in the %Ndfa between the organic and the conventional system was significant when the preceding crop was cabbage or pea (Table 6). The shoot dry biomass (SDB) was not affected by the farming system. As a rule, the dry shoot biomass (SDB) of faba bean was higher when the preceding crop was a legume compared to cabbage in both EYs, although some differences were insignificant. In the first EY, the total-N concentration in the shoot of faba bean was similar in all treatments, while in the second EY, it was significantly lower when the preceding crop was cabbage, compared to pea in the organic farming system and to faba bean in the conventional farming system.

Table 5. Impact of the farming system (organic or conventional) and the preceding crop (cabbage, pea, and faba bean: C, P, and F, respectively) on the percentage of nitrogen derived from atmosphere, shoot dry biomass, shoot total N (%), and total N fixed biologically by faba bean (BNF) at the first EY (2015–2016).

Farming System	Preceding Crop	Ndfa (%)	SDB (t ha^{-1})	Total N (%)	BNF (kg ha^{-1})
Organic	C-F	69.35 ± 4.55 ^{b}	3.16 ± 0.06 ^{b}	3.55 ± 0.09	77.80 ± 0.82 ^{d}
	P-F	78.61 ± 3.51 ^{a}	3.91 ± 0.07 ^{a}	3.61 ± 0.06	110.96 ± 1.05 ^{a}
	F-F	77.89 ± 1.72 ^{a}	3.66 ± 0.03 ^{ab}	3.53 ± 0.11	100.63 ± 1.44 ^{b}
Conventional	C-F	71.63 ± 4.71 ^{b}	3.36 ± 0.08 ^{b}	3.29 ± 0.03	79.18 ± 0.56 ^{d}
	P-F	68.24 ± 4.18 ^{b}	3.80 ± 0.08 ^{a}	3.52 ± 0.07	91.28 ± 0.83 ^{c}
	F-F	70.18 ± 2.47 ^{b}	3.74 ± 0.04 ^{a}	3.39 ± 0.08	88.98 ± 0.79 ^{c}
Main effects					
Organic		75.28 ± 1.78	3.58 ± 0.08	3.56 ± 0.07 ^{a}	96.46 ± 4.29
Conventional		70.02 ± 2.61	3.63 ± 0.07	3.40 ± 0.03 ^{a}	86.48 ± 1.67
C-F		70.49 ± 2.08	3.26 ± 0.05	3.42 ± 0.06 ^{a}	78.49 ± 0.52
P-F		73.43 ± 1.16	3.86 ± 0.04	3.57 ± 0.09 ^{a}	101.12 ± 3.78
F-F		74.04 ± 1.35	3.70 ± 0.03	3.46 ± 0.08 ^{a}	94.81 ± 2.39
Statistical significance					
Farming system (FS)		**	ns	ns	ns
Preceding crop (PC)		*	*	ns	*
FS × PC		**	**	ns	**

Values are means (n = 4) ± standard errors. Means within the same column followed by different letters indicate significant differences according to the Duncan's multiple range test: * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively; ns = not significant. %Ndfa: % nitrogen derived from atmosphere; SDB: shoot dry biomass; Total N: total nitrogen (%); BNF: biological nitrogen fixation (total N fixed biologically).

Finally, the total weight of N per cultivated area unit that was fixed biologically by faba bean (BNF) was significantly lower when the preceding crop was cabbage compared to the legumes in both EYs and cropping systems. When comparing the two legumes as preceding crops, the BNF was significantly higher with pea in the organic system but similar to both legumes in the conventional system.

Table 6. Impact of the farming system (organic or conventional: O or C, respectively) and the preceding crop (cabbage, pea, and faba bean: C, P, and F, respectively) on the percentage of nitrogen derived from atmosphere, shoot dry biomass, shoot total N (%), and total N fixed biologically by faba bean (BNF) at the second EY (2016–2017).

Farming System	Preceding Crop	Ndfa (%)	SDB (t ha^{-1})	Total N (%)	BNF (kg ha^{-1})
Organic	C-F	83.45 ± 1.68 ^{b}	3.41 ± 0.04 ^{c}	3.18 ± 0.09 ^{b}	90.49 ± 0.82 ^{c}
	P-F	93.83 ± 0.076 ^{a}	4.28 ± 0.02 ^{a}	3.66 ± 0.06 ^{a}	146.98 ± 1.24 ^{a}
	F-F	89.12 ± 1.72 ^{ab}	3.89 ± 0.04 ^{b}	3.22 ± 0.12 ^{b}	111.63 ± 0.87 ^{b}
Conventional	C-F	76.23 ± 1.32 ^{c}	3.62 ± 0.03 ^{bc}	3.31 ± 0.09 ^{b}	91.34 ± 0.63 ^{c}
	P-F	84.13 ± 3.10 ^{b}	3.97 ± 0.04 ^{b}	3.45 ± 0.16 ^{ab}	115.23 ± 0.75 ^{b}
	F-F	82.53 ± 3.14 ^{bc}	3.92 ± 0.03 ^{b}	3.63 ± 0.13 ^{a}	117.44 ± 0.86 ^{b}
Main effects					
Organic		88.80 ± 0.93	3.86 ± 0.10	3.35 ± 0.08	116.37 ± 7.05
Conventional		80.96 ± 1.51	3.84 ± 0.06	3.46 ± 0.08	108.00 ± 3.63
	C-F	79.84 ± 1.00	3.52 ± 0.03	3.25 ± 0.06	90.92 ± 0.49
	P-F	88.98 ± 1.65	4.13 ± 0.06	3.56 ± 0.11	131.11 ± 5.97
	F-F	85.83 ± 1.91	3.91 ± 0.02	3.43 ± 0.10	114.53 ± 1.24
Statistical significance					
Farming system (FS)		*	ns	ns	ns
Preceding crop (PC)		*	**	*	**
FS × PC		*	*	*	**

Values are means (n = 4) ± standard errors. For each factor, or for all factorial combinations when the interaction was significant, means within the same column followed by different letters indicate significant differences according to the Duncan's multiple range test: * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively; ns = not significant. %Ndfa: % nitrogen derived from atmosphere; SDB: shoot dry biomass; Total N: total nitrogen (%); BNF: biological nitrogen fixation (total N fixed biologically).

3.6. Yield Components, Pod and Green Seed Yield

The length of faba bean pods was not influenced by the farming system or the preceding crop in both EYs (Table 7). The number of faba bean pods per plant (PN) and the total fresh pod yield (TFPY) were not influenced by the farming system in both EYs. Both the PN and TFPY were significantly higher with legumes as preceding crops compared to cabbage in both cropping systems and EYs. When comparing the two legumes as preceding crops, pea resulted in higher PN and TFPY compared to faba bean in the organic farming system, while in the conventional system, both legumes rendered similar PN and TFPY.

Table 7. Impact of the farming system (organic or conventional: O or C, respectively) and the preceding crop (cabbage, pea, and faba bean: C, P, and F, respectively) on yield parameters of faba bean in both EYs.

		EY 2015–2016			EY 2016–2017		
Farming System	Preceding Crop	PL (cm)	PN (No Plant^{-1})	TFPY (t ha^{-1})	PL (cm)	PN (No Plant^{-1})	TFPY (t ha^{-1})
Organic	C-F	7.38 ± 0.21	35.82 ± 1.22 ^{c}	26.63 ± 0.74 ^{c}	7.64 ± 0.19	38.40 ± 0.71 ^{d}	23.60 ± 0.66 ^{c}
	P-F	6.91 ± 0.19	46.70 ± 0.48 ^{a}	34.17 ± 0.79 ^{a}	7.54 ± 0.21	45.45 ± 0.79 ^{a}	32.14 ± 0.73 ^{a}
	F-F	6.58 ± 0.22	42.47 ± 0.62 ^{b}	30.12 ± 0.81 ^{b}	7.86 ± 0.22	40.60 ± 0.42 ^{c}	28.13 ± 0.42 ^{b}
Conventional	C-F	7.16 ± 0.19	37.62 ± 0.84 ^{c}	27.51 ± 0.83 ^{c}	7.43 ± 0.20	38.97 ± 0.61 ^{d}	24.12 ± 0.79 ^{c}
	P-F	6.79 ± 0.23	41.57 ± 0.83 ^{b}	30.70 ± 0.88 ^{b}	7.13 ± 0.21	42.52 ± 0.67 ^{b}	28.72 ± 0.45 ^{b}
	F-F	6.70 ± 0.21	42.07 ± 0.78 ^{b}	30.88 ± 0.84 ^{b}	7.48 ± 0.23	42.85 ± 0.66 ^{b}	27.86 ± 0.44 ^{b}

Table 7. *Cont.*

Farming System	Preceding Crop	EY 2015–2016 PL (cm)	EY 2015–2016 PN (No Plant^{-1})	EY 2015–2016 TFPY (t ha^{-1})	EY 2016–2017 PL (cm)	EY 2016–2017 PN (No Plant^{-1})	EY 2016–2017 TFPY (t ha^{-1})
Main effects							
Organic		6.91 ± 0.20 ^{a}	41.66 ± 1.36	30.31 ± 1.02	7.68 ± 0.21 ^{a}	41.48 ± 0.96	27.95 ± 1.21
Conventional		6.88 ± 0.21 ^{a}	40.42 ± 0.69	29.67 ± 0.63	7.35± 0.22 ^{a}	41.45 ± 0.63	26.90 ± 0.66
C-F		7.27 ± 0.22 ^{a}	36.72 ± 0.75	27.07 ± 0.54	7.54 ± 0.20 ^{a}	38.69 ± 0.44	23.86 ± 0.51
P-F		6.85 ± 0.21 ^{a}	44.14 ± 0.99	32.44 ± 0.87	7.33 ± 0.23 ^{a}	43.99 ± 0.76	30.43 ± 0.88
F-F		6.63 ± 0.22 ^{a}	42.27 ± 0.49	30.50 ± 0.55	7.67 ± 0.21 ^{a}	41.73 ± 0.49	28.00 ± 0.29
Statistical significance							
Farming system (FS)		ns	ns	ns	ns	ns	ns
Preceding crop (PC)		ns	***	***	ns	***	***
FS × PC		ns	**	**	ns	**	**

Values are means (n = 4) ± standard errors. For each factor, or for all factorial combinations when the interaction was significant, means within the same column followed by different letters indicate significant differences according to the Duncan's multiple range test: ** and *** indicate significance at $p < 0.01$ and $p < 0.001$, respectively; ns = not significant. PL: pod length; PN: pod number; TFPY: total fresh pod yield.

4. Discussion

4.1. Contribution of Legumes as Preceding Crops in Rotations

Legume crops constitute essential components of rotations in organic farming systems due to their ability to provide plant-available nitrogen to agricultural ecosystems through symbiotic N_2 fixation [1,26]. However, many factors, including the farming system, the genotype, the preceding crop, and the soil organic and inorganic N status, can be crucial for the net input of plant-available N to agroecosystems through biological N_2 fixation. In the present study, the interactive effects of the farming system (organic or conventional) and the preceding crop were tested in a baba bean crop, using the same genotype and starting with the same soil N status.

Crop rotation has been used for thousands of years as an essential cultural practice for controlling pests and diseases and maintaining soil fertility in agriculture [27]. The tremendous progress with the production of synthetic fertilisers, pesticides, and insecticides during the fifties led to the feeling that rotation is no longer needed in agriculture [28]. However, the development of resistant pests and pathogens to pesticides, the negative environmental impacts of the extensive fertiliser use, including the greenhouse gas (GHG) emissions for industrial fixation of N_2, and the expansion of cropping systems relying on minimal or even no use of synthetic agrochemicals, make the rotation more actual than ever. In the current study, we examined only a part of a potential rotation sequence, testing the impact of the preceding crop on faba bean cultivation. Rotation constitutes an essential component of organic farming systems and, thus, it is more important for organic than for conventional farming systems [26]. Therefore, in the current study, we tested the same crop sequences in both an organic and a conventional farming system. However, in our study, the faba bean crop was not faced with any infection by pests or pathogenic microorganisms. Thus, the liberty to use synthetic pesticides or insecticides in the conventional farming system did not provide any benefit to the faba bean plants grown conventionally over those grown organically.

4.2. N Nutrition of Faba Bean and Its Impact on Yield

The higher soil NO_3 concentration in the conventional compared to the organic farming system, especially in the second EY, is associated with the supply of synthetic N fertiliser to the plants in this cropping system. However, the difference in NO_3 availability between the two cropping systems was not large because faba bean is characterised by a high N_2-fixing efficiency [20,29]. As a result, faba bean is capable of fully covering its N needs through biological N_2 fixation [30]. Therefore, in the current study, the pod yield was similar in the organic and the conventional system, despite the minor differences in soil NO_3 availability, especially at crop establishment. In contrast to the mineral N, the organic N was higher in the organic farming system due to manure application in the latter. The plant residues were left in the field in both cropping systems, and, thus, they are not responsible for the differences in soil total N between the two cropping systems. The higher levels of soil total-N before establishing the faba bean crop when the preceding crop was pea or faba bean is ascribed to the higher mass of plant residues left by these two legume species to the soil after crop termination, compared to cabbage [31].

The higher pod yield obtained when faba bean was grown after either pea or faba bean compared to cabbage as the preceding crop correlates well with the higher soil NO_3-N levels measured in these plots. Since this was observed in both cropping systems, it cannot be ascribed to differences in fertilisation but rather to differences in NO_3 originating from mineralisation of the crop residues. Both pea and faba bean leave substantially higher crop residues in a field compared to cabbage [32]. Thus, they have a higher capacity to supply plant-available N to the next crop through mineralization of their residues left to the field after the harvest. This consideration also agrees with the higher total N concentrations in the soil when faba bean followed a pea or a faba bean crop compared to cabbage. In addition to the quantity, differences in the tissue N content and the texture of the organic matter, which determines the mineralisation rate [33] also have a strong impact on the contribution of crop residues to the supply of a crop with plant-available N. The study of organic N fractions and their transformations over time may be a useful tool to refine the estimations about the N availability for crops, estimate the supply of plant available N to the soil, and evaluate the potential release of mineral N by organic fertilisers [34].

4.3. Soil and Plant Tissue Potassium

The significantly lower soil K concentration in the organic farming system in both EYs is ascribed to the lower amounts of K supplied to this cropping system via fertilisers, as organic is a low-input cropping system. Other researchers [35], comparing crop rotations in organic and conventional farming system, found that, regardless of the plant species (potato, winter wheat, field bean, and spring barley) in the crop rotations, the conventional farming system contributed to a significant increase in soil phosphorus and potassium content, on average, by 7%. The lower K concentration in the epigeous tissues of faba bean grown organically is reasonably ascribed to the lower soil K levels in this farming system. However, the K concentrations measured in the epigeous tissues of faba bean grown organically, albeit significantly lower than in conventionally grown plants, were within the optimal range for faba bean. Indeed, as reported by Aini and Tang [36], the critical levels of tissue K concentrations associated with K deficiency are 13 to 15 mg g^{-1} at the 7- to the 8-leaf stage in youngest fully expanded leaf (YFEL), 11 to 12 mg g^{-1} in the first plus second leaf blades below the YFEL, and 18 to 20 mg g^{-1} in the whole shoot of faba bean. Hence, the differences in soil K levels and concomitantly in tissue K concentrations between the organic and the conventional farming system, albeit significant, were not large enough to significantly reduce plant biomass or yield.

As a general trend, significantly higher soil K concentrations were observed when faba bean followed cabbage, compared to faba bean itself or pea. This is mainly ascribed to the higher amount of K fertiliser applied to cabbage compared to that delivered to the two legume species, following the common commercial practice. However, it can be partly ascribed to the higher input of K to the soil through the crop residues of cabbage compared to those of the two legumes, as the standard K concentrations in the tissues of cabbage are substantially higher than the respective concentrations in pea and faba bean. Indeed, the levels of K in the tissues of plants supplied optimally with N is mainly determined by the botanical family. As reported by Greenwood et al. [37] the tissue K levels in the leaf dry matter (including stems) of crops optimally fertilized with K at harvest were generally between 9 and 11 mg g^{-1} in *Amaryllidaceae* plants, 11 and 12 mg g^{-1} in Fabaceae plants and 19 to 25 mg g^{-1} in plants of the Brassicaceae family.

4.4. Root Nodulation

The number of nodules per volume of soil (No L^{-1}) in the roots of organically grown faba bean was significantly higher when the preceding crop was also faba bean, compared to cabbage or pea as a preceding crop. This is ascribed to the presence of a higher native population of rhizobia nodulating faba bean in the soil when faba bean is grown after faba bean, as in that case the preceding crop is a host of the N_2-fixing rhizobia strains that nodulate faba bean. As reported by Efstathiadou et al. [38], the vast majority of isolates obtained from local soils belong to genospecies gsF-2, represented by the type strain *Rhisobium laguerreae*, and are characterized by a high N_2-fixing efficiency. Furthermore, the faba bean landrace used in the current study was 'AUALEFKADAfb001', which was selected because of its higher efficiency to form nodules with rhizobia and symbiotically fix high amounts of N_2 [20].

4.5. Biological Nitrogen Fixation

In the current study, an interaction was observed regarding the percentage of nitrogen derived from atmosohere *(Ndfa%).* The highest *Ndfa%* was recorded when faba bean and pea were the preceding crops compared to cabbage in the organic farming system, while lower values were recorded in the conventional farming system, regardless of the preceding crops. The highest BNF value was recorded when pea was the preceding crop in the organic farming system. However, in the conventional farming system, the use of both pea and faba bean as preceding crops rendered higher levels of BNF than the use of cabbage, while there was no significant difference between the two legumes used as preceding crops. In both EYs, BNF tended to be higher when faba bean was grown organically compared to conventional cropping, but the differences were statistically insignificant, in agreement with a previous report [20]. Collino et al. (2015) [25], reported that the BNF efficiency of a crop is influenced not only by the presence of suitable rhizobia in the soil and the genotype of the legume but also by several environmental factors. One of these factors is the availability of inorganic N in the root zone of legumes. It is well known that high levels of inorganic N in the root zone inhibit rhizobial nodulation in various legume crops [39–41]. Thus, the lower number of nodules and the lower %Ndfa in the conventional compared to the organic farming system are ascribed to the supply of inorganic N and especially NO_3-N in the conventionally treated plots but not in those treated organically.

In the current study, the average amount of atmospheric N fixed by faba bean (BNF) in both EYs was 106.4 kg ha^{-1} in the organic crop and 97.24 kg ha^{-1} in the conventional crop of faba bean, with a mean of 101.8 kg ha^{-1} in both farming systems. This level of BNF is sufficiently high, confirming previous reports about the high BNF efficiency of faba bean [20]. In a field-scale study conducted in the British Isles over several consecutive seasons, Maluk et. al. [30] observed that faba bean was capable of covering almost all of its N requirements through biological N_2 fixation under the relatively wet and cool climate of that region. Jensen et. al. [42] reported a range of 73–211 kg N ha^{-1} yr^{-1} for N fixed by faba bean. In another study by Oliveira et. al. [43], the three-year average N fixation recorded for faba bean was 41 kg N ha^{-1} yr^{-1}. The higher BNF values estimated in the current study appear to be a consequence of the high %*Ndfa* levels, which are partly a consequence of the high BNF efficiency of the local faba bean landrace used in the current experiments [20].

5. Conclusions

The results of the current study showed that:

- Pea and faba bean as previous crops can increase nitrate concentrations in the soil before planting and at the early stages of the next crop as compared to cabbage as preceding crop, especially in organic farming systems;
- When faba bean is not affected by diseases, growing conventionally faba bean again after faba bean for a second year does not decrease yield compared to pea as preceding crop;

- In organic farming, faba bean cultivation after faba bean decreases the yield compared to rotating pea with faba bean, although the cultivation of faba bean after faba bean does not decrease its efficiency to biologically fix N_2 compared to pea as preceding crop;
- Peas and faba beans could preferably be used in crop rotation schemes with vegetables (e.g., Brassicaceae) in organic farming systems.

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