Comparative Assessment of Different Crop Rotation Schemes for Organic Common Bean Production

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Abstract: The aim of the current study was to contribute to the establishment of sustainable organic crop rotation schemes for common bean under mild-winter climatic conditions. Common bean was cultivated according to organic or conventional farming practices during spring-summer in two successive years with crop and treatment during the preceding winter as either: (a) organic broccoli, (b) conventional broccoli, (c) organic faba bean used as green manure, or (d) fallow. Common bean was either inoculated with *Rhizobium tropici* CIAT 899 or non-inoculated, while faba bean was inoculated or non-inoculated with *Rhizobium laguerreae* VFLE1. Inoculating faba bean with rhizobia enhanced dry biomass production and biological N-fixing ability in both experimental years. Furthermore, organic farming did not restrict the yield of broccoli compared to conventional practices during the first year, while the reverse was the case in the second year, due to reduced soil N availability. Furthermore, green manure enhanced the fresh pod yield in the following organic crop of common bean in both years. The lowest yield was recorded in organically grown common bean when the preceding winter crop was organically grown broccoli in both years. *Rhizobia* inoculation of the common bean during the first year slightly increased atmospheric N fixation by common bean.

Keywords: organic farming; faba bean; *Phaseolus vulgaris* sp.; biological nitrogen fixation (BNF); rhizobia; nitrogen availability; conventional farming; broccoli

1. Introduction

Organic agriculture relies on more environment-friendly farming practices, including the use of green manure, intercropping, and crop rotation that improve soil structure, soil fertility, and crop quality. In addition, organic farming provides a more favorable proportion of energy input/output [1]. This is due to the low input of mineral fertilizers (P, K), while the use of synthetic N-fertilizer, pesticides, and herbicides is fully prohibited [2]. The advantages of organic agriculture in both environment and food security have given rise to strong research interest aiming to render this cropping system a viable alternative to conventional farming [3–6]. However, this depends on the ability of organic farming to compete with the productivity of conventional systems.

On the other hand, organic agriculture is considered an ineffective approach to food availability [1,7]. Furthermore, in order for organic agriculture to achieve high yield compared to conventional practices, more arable farmland is often required, thereby risking the loss of semi-natural ecosystems, such as
woodland [8,9]. According to several studies, the average yield in organic agriculture is 8% to 33% lower compared to that obtained from conventional farming systems [10].

Nitrogen (N) is a macronutrient required at relatively high amounts by plants to increase yields to economically sustainable levels. In organic agriculture, N is one of the key factors limiting productivity as its supply in the form of inorganic fertilizers is unacceptable [11,12]. N supply to organic farming systems is mainly achieved through renewable organic sources such as legumes used as green manure, animal-manures, compost, and crop rotation schemes comprising N-fixing crops followed by N-utilizing crops. These sources should be capable of providing similar amounts of N in organic farming to those provided by conventional farming and the addition of synthetic N-fertilizers [13]. However, N from organic N-fertilizer sources is unavailable to plants immediately after its application and is released gradually through mineralization catalyzed by enzymes of soil microorganisms. Therefore, in organic crops, the well-timed N supply is more important than the total amount of N applied. If sufficient N is not available to the plants at critical plant developmental stages, the growth and yield would be restricted [14].

Legumes cultivated in crop rotation schemes as green manure are anticipated to improve N availability of the subsequent non-legume crop. This beneficial effect of legumes is mainly due to their ability to fix atmospheric N₂ through their symbiosis with nitrogen-fixing bacteria (rhizobia). Faba bean is a common crop cultivated as green manure due to its high N-fixing activity, as indicated by the high percentages of N derived through the atmosphere (% Ndfa) that have been estimated for this plant, which under no N inputs, can reach levels up to 99% [15]. Furthermore, green manure provides additional benefits, such as suppressing weed and disease occurrence, plus reduced leaching of nutrients from the soil [15]. However, the N-fixing activity of the faba bean is dependent on the availability of compatible rhizobia in the soil, climatic conditions, and soil fertility [16]. According to Denton et al. [17], inoculation of faba bean plants with effective rhizobia can enhance its N-fixing activity. However, this also depends on the population of rhizobia in the soil, according to Heridge et al. [18], as low populations could restrict N fixation by the host legume, even if the rhizobia species is effective.

Common bean (Phaseolus vulgaris sp.) is the legume crop most commonly cultivated globally for human food production due to the high nutritional and organoleptic value of its pods and seeds [19,20]. Furthermore, common bean is widely cultivated also for the production of fresh pods consumed as a vegetable [21]. Even though common bean, as a legume, can readily nodulate and fix atmospheric N₂ through symbiosis with N-fixing bacteria, its N-fixing ability is relatively low compared to that of other commonly cultivated grain legume species [22]. Thus, the growth of common bean and the achievement of high yields is largely dependent on additional N supply through fertilization [23]. The N-fixing activity can be restricted if the plant-available N in the soil after crop establishment is inadequate. Thus, the levels of added inorganic N in soil need efficient management to optimize the trade-off with biological N fixation. For example, some inorganic N could be available to plants during their initial growth stages, and until rhizobia form nodules that efficiently fix atmospheric N₂. Compared to conventional agriculture, organic cropping systems are characterized by low availability of mineral N. However, the application of slow-release N fertilizers can provide a positive effect on crop establishment and later N-fixing activity [24,25]. Finally, the lack of other nutrients beyond N in long-term organic crop may also restrict the N-fixing activity [26].

Common bean is frequently cultivated as a warm-season vegetable during spring and summer in open fields in the northern hemisphere. However, in countries with mild climatic conditions such as those prevailing in the Mediterranean basin, it is usual to cultivate a cold season vegetable crop during the winter, in the same field that was used for common bean production during the warm season. Alternatively, and especially in fields cultivated organically with common bean, the winter may be utilized to establish a green manure crop aiming to increase the soil fertility for the subsequent bean crop.

Taking the above into consideration, the main objective of the present investigation was to test the impact of the preceding winter crop on spring-summer cultivation of the common bean in organic
farming systems under mild climatic conditions allowing for winter cultivation. The standard practices during the preceding winter in fields cultivated organically with the common bean in spring-summer are either left fallow or cultivation of a cold-season legume (e.g., vetch or faba bean) during the winter, which is incorporated into the soil as green manure. In the current study, in addition to these standard options, the possibility to cultivate organically a cold-season non-legume vegetable during the winter as a preceding crop to a spring-summer cultivation of the common bean was investigated. The rotation of a winter non-legume vegetable with a spring-summer common bean crop during the same year was tested following, not only organic but also conventional farming practices, which served as a control. Finally, in all rotation treatments, there were plots with common beans inoculated or non-inoculated with *Rhizobium tropici* to test whether rhizobia inoculation provides a benefit to the crop. To obtain results that are more credible and test the long-term effects of organic fertilization on organic crops, the cropping sequence was repeated over two years (cropping seasons) in the same field.

2. Materials and Methods

The field experiment was conducted during autumn 2017 to summer 2018 and repeated the following year (autumn 2018 to summer 2019) at the experimental facilities of the Laboratory of Vegetable Production in the Agricultural University of Athens (37°58’56.4” N 23°42’15.7” E). The total area of the experimental field, which extended to 375 m², was divided into 32 farming plots, 5 m² each. The crops were established on a sandy loam field according to the presented physical properties of the experimental field (Table 1), which had remained uncultivated for the last five years before the onset of the current research. As the experimental field was uncultivated during the last five years, no microbial characterization of the soil was performed. In addition, climatic data, particularly monthly temperature (minimum, average minimum, maximum, and average maximum) and monthly mean precipitation of the first and second experimental year, are presented in Figures 1 and 2, respectively.

![Figure 1](image-url)  
**Figure 1.** Monthly maximum (Tmax), average maximum (MTmax), average minimum (MTmin), and minimum (Tmin) and precipitation (RR) during the 1st experimental year (September 2017–August 2018) in Athens, Greece.
Table 1. Physical and chemical properties of the soil at the experimental field.

| Parameter       | Value     | Parameter       | Value               |
|-----------------|-----------|-----------------|---------------------|
| Clay            | 20%       | Total N         | 0.2%                |
| Silt            | 14%       | Available P     | 153.5 mg kg⁻¹       |
| Sand            | 66%       | Exchangeable K  | 478 mg kg⁻¹         |
| pH              | 7.7       | Organic matter  | 5%                  |
| Electrical conductivity | 710 µS cm⁻¹ | Total CaCO₃   | 15.98%              |

Figure 2. Monthly maximum (Tmax), average maximum (MTmax), average minimum (MTmin), and minimum (Tmin) and precipitation (RR) during the 2nd experimental year (September 2018–August 2019) in Athens, Greece.

2.1. Winter Cultivation Period: Experimental Set up and Fertigation Scheme

During the autumn-winter cultivation periods 2017–2018 and 2018–2019, four different cultivation practices were applied in the experimental plots as follows: (1) organic cultivation of broccoli, (2) conventional cultivation of broccoli, (3) organic cultivation of faba bean, which was incorporated into the soil as green manure, or (4) left fallow. In addition, the faba bean used as green manure was divided into two sub-treatments, particularly inoculation (3a) or non-inoculation (3b) with the rhizobium strain *Rhizobium laguerreae* VFLE1.

A one-head broccoli (*Brassica oleracea* var. *italica*) hybrid (‘Monrello’) was cultivated both conventionally and organically at a plant density of six plants m⁻². To apply green manure, a local landrace originating from the Greek island Lefkada was used to establish the faba bean (*Vicia faba* L.) crop at a plant density of 30 plants m⁻². This crop was either inoculated with *Rhizobium laguerreae* VFLE1 or non-inoculated (treatments 3a and 3b, respectively). The treatments of organic and conventional broccoli and fallow were replicated eight times, while the treatments of faba bean used as green manure (inoculated or non-inoculated) were replicated four times. Besides, during the seedling’s preparation of broccoli plants, standard conventional and organic farming practices were applied to the plants, which were destined for conventional and organic farming of broccoli, respectively. At the end of the winter cultivation period of both experimental years, the plant residues of the above crops were incorporated into the soil by plowing at a depth 20 cm.
During the first autumn-winter cultivation period, the broccoli seedlings were transplanted on 15 October 2017 in both the organically and the conventionally cultivated plots. On the same date, faba bean seeds inoculated or non-inoculated with *Rhizobium laguerreae* VFLE1 were sown in the respective plots. In the plots left fallow during autumn-winter, no action was taken, and the natural vegetation was left to grow freely during the whole cultivation period. Both the organic and conventional broccoli crops were harvested four times during 1–15 January 2018, when they reached commercial maturity according to standard practices in commercial crops. The faba bean plants were incorporated into the soil as green manure on 15 February 2018, when they reached their 50% flowering stage. On the same day, the plant residues in the broccoli plots as well those left fallow were also incorporated into the soil. Finally, on 5 March 2018, the field was ready for the subsequent spring-summer (1st) cultivation period. During the second experimental year, the broccoli and faba bean crops were established on 30 October 2018 by planting seedlings as well as inoculated and non-inoculated faba bean seeds, respectively. The broccoli crop, irrespective of the applied farming practices, was harvested four times during 15–30 January 2019. The faba bean plants reached their 50% flowering stage on 28 February 2019, and the plant residues of the different crops in the second winter cultivation period were incorporated into the soil on 5 March 2019. During incorporation, plant residues were cut with a flail mower, and then the chopped crop residues were incorporated into the soil by plowing. After incorporation of the plant residues, the field was re-plowed two times using a rotary cultivator (one per week) to facilitate deeper incorporation of the plant biomass to the soil, especially in the green manure plots. Finally, on 30 March 2019, the field was ready for the subsequent (2nd) summer cultivation period.

The amounts of nutrients applied in each treatment were set according to common cultural practices. In the organic cultivation of broccoli, the applied basal dressing during the first experimental year included the addition of sheep manure, which provided 90 kg ha$^{-1}$ N, and Patent kali (30% K$_2$O, 10% MgO and 42.5% SO$_3$, K+5 AG) at a rate of 400 kg ha$^{-1}$. The concentrations of the main nutrients in the sheep manure, which originated from certified organic farms according to Council Regulation (EC) No 834/2007, were as follows: 0.84% total-N, 0.3% P$_2$O$_5$, 0.7% K$_2$O, 0.38% CaO, and 0.24% MgO, on dry weight (DW) basis. In addition to the basal dressing, the organic broccoli crop was fertilized with an organic fertilizer (7-4-7+2MgO+0.2B), which provided 40 kg ha$^{-1}$ N. In the conventional broccoli crop, the basal dressing included an inorganic fertilizer (11-15-15), which provided 132 kg ha$^{-1}$ N, 180 kg ha$^{-1}$ P and 180 kg ha$^{-1}$ K. Furthermore, the conventionally cultivated broccoli plots were fertigated with a nutrient solution (9.6 mmol L$^{-1}$ N, 5 mmol L$^{-1}$ K), which provided 56.4 kg ha$^{-1}$ N throughout the whole cultivation period.

During the second experimental year, basal dressing in organic broccoli included the addition of N as sheep manure at a rate of 120 kg ha$^{-1}$, and potassium as patent kali at a rate of 400 kg ha$^{-1}$, while no fertilizers were applied until the end of the cultivation period. In the conventionally cultivated broccoli, the basal dressing consisted of the addition of an inorganic fertilizer, which provided 79.2 kg ha$^{-1}$ N, 108 kg ha$^{-1}$ P, and 108 kg ha$^{-1}$ K. In addition to the basal dressing, the conventionally cultivated broccoli was fertigated, during the whole cultivation period with a nutrient solution (9.6 mmol L$^{-1}$ N, 5 mmol L$^{-1}$ K), which provided 160 kg ha$^{-1}$ N.

The total N input during the first winter-cultivation period was 130 kg ha$^{-1}$ N in the organic broccoli and 188.4 kg ha$^{-1}$ N in the conventional broccoli. During the second winter cultivation period; however, the total N input changed to 120 kg ha$^{-1}$ N in the organic broccoli and 239.2 kg ha$^{-1}$ N in the conventionally grown broccoli. The increased supply of N in the conventional broccoli during the second, compared to the first experimental year, was due to the higher fertigation requirements during the second year because of less precipitation during the cropping period. As a too low soil N availability during the early vegetative stage may restrict rhizobia nodulation in legume crops [27], the plots intended for faba bean cultivation were fertilized with sheep manure, which provided 40 kg ha$^{-1}$ N, and patent kali at a rate of 400 kg ha$^{-1}$ in both winter cultivation periods. Finally, all the experimental
crops were drip-irrigated during both autumn-winter experimental periods, whenever the natural precipitation was incapable of covering the crop watering needs.

2.2. Summer Cultivation Period: Experimental Set up and Fertigation Scheme

During the subsequent spring-summer cultivation period, a climbing variety of common bean (*Phaseolus vulgaris* cv. Borlotto) was cultivated. In the plots with treatments 1, 3a, 3b, and 4 during the winter, organic farming practices were applied in the subsequent common bean crop, while in treatment 2, the subsequent common bean crop was treated according to conventional farming practices (Table 2). In treatments 1, 2, and 4 during autumn-winter 2018–2019, the subsequent common bean crop was either inoculated with *Rhizobium tropici* CIAT 899 or not. In the plots of faba bean inoculated with *Rhizobium laguerreae VFLE1* (3a), the seeds of the common bean were also inoculated with *R. tropici* CIAT 899. Similarly, in the plots of faba bean that were not inoculated with *Rhizobium laguerreae VFLE1* (3b), the seeds of common bean were not inoculated with *R. tropici* CIAT 899. The plant density of common bean was six plants m⁻². An overview of the treatments during both cultivation periods is provided in Table 2.

| Treatments                      | Replicates | Winter Cultivation Period | Summer Cultivation Period |
|---------------------------------|------------|----------------------------|---------------------------|
| Organic broccoli (1)            | 8          | Inoculated                | Organic common bean       |
|                                  |            | Non-inoculated            | 4                         |
| Conventional broccoli (2)       | 8          | Inoculated                | Conventional common bean  |
|                                  |            | Non-inoculated            | 4                         |
| Faba bean Inoculated (3a)       | 4          | Inoculated                | Organic common bean       |
| Faba bean non-inoculated (3b)   | 4          | Non-inoculated            | 4                         |
| Fallow (4)                      | 8          | Inoculated                | Organic common bean       |
|                                  |            | Non-inoculated            | 4                         |

During the first summer cultivation period, the organic and conventional common bean crop was established at the experimental field on 20 March 2018. Moreover, the common bean plants reached the 50% flowering stage on 30 April 2018, while the harvesting period commenced on 10 May 2018 and was terminated on 30 June 2018. The bean crop for the second summer cultivation period was established on the experimental field on 25 April 2019. Furthermore, the 50% flowering stage of the common bean plants was recorded on 5 June 2019. Finally, the crop was harvested during 15 June 2019 and 5 August 2019. All plants during both spring-summer experimental periods were drip-irrigated using separated irrigation networks for the organic and conventional plots.

In the organically cultivated common bean, the basal dressing included sheep manure, which provided 40 kg ha⁻¹ N, and 750 kg ha⁻¹ Patentkali. Moreover, no other fertilizers were applied at the organically cultivated plots until the end of the cultivation period. In the conventionally grown common bean crop, inorganic fertilizer (11-15-15) was applied as basal dressing, which provided 55 kg ha⁻¹ N. Additionally, at the stage of 50% flowering, a nutrient solution (3 mmol L⁻¹ N and 5.1 mmol L⁻¹ K) was applied through the drip irrigation system to the conventional common bean crop, which provided 20 kg ha⁻¹ N. Consequently, the total amount of applied N was 40 kg ha⁻¹ in the organic and 75 kg ha⁻¹ in the conventional farming system, respectively. Finally, in all the experiments during both years, only plant protection practices permitted in organic agriculture were used, while weeds were always controlled through hoeing.
2.3. Sampling, Measurements, and Methods

2.3.1. Inoculation with Rhizobia

For the inoculation of faba bean seeds, the indigenous strain VFLE1 of *Rhizobium laguerreae* was used, which was isolated by the Laboratory of General and Agricultural Microbiology of AUA (Efstathiadou et al., unpublished data) from nodules of field-grown faba bean (landrace ‘Lefkada’). To inoculate faba bean, the seeds were pelleted with Arabic gum and then soaked into a liquid culture (10⁹ cfu/mL) of the above strain. Finally, the inoculated seeds were sown in a well-moistened soil.

To inoculate common bean, the commercial strain *Rhizobium tropici* CIAT 899 was selected, which originates from Brazil. The common bean seeds were placed in a temperature-controlled (25 °C) incubation chamber for germination. The germinated seeds were soaked in liquid culture (10⁹ cfu/mL) of the above strain, and then transplanted in plastic sowing trays using turf as substrate. At the three-leaves stage, the plants were transplanted into the experimental field.

2.3.2. Growth and Yield Parameters

At the 50% flowering stage, the fresh weight of shoot (g plant⁻¹) of both faba (five plants per experimental plot) and common bean (three plants per experimental plot) plants were recorded. The dry weight (g plant⁻¹) was estimated by drying the samples in a forced-air oven at 70 °C for five days.

To evaluate the yield of broccoli under the different cultivation systems, the heads of broccoli were harvested at the marketable size, and the fresh weight (g plant⁻¹) and dry matter content (%) of the heads were recorded. The harvesting of common bean commenced when the fresh pods reached marketable size and repeated weekly. In total, the common bean crops were harvested nine times during the first experimental year and eight times during the second experimental year. To assess the yield performance of the common bean crops, the fresh pod weight (g plant⁻¹), number of pods (N plant⁻¹), and the mean fresh weight (g pod⁻¹) were assessed.

2.3.3. Soil Analyses

Soil samples from all the experimental plots were collected at a depth of 0–15 cm after planting at flowering and at the reproductive stage of growth. More particular, during the first winter cultivation period, soil samples were collected during broccoli flower heads formation, at the end of the broccoli cultivation period and during the 50% flowering stage of faba bean plants (i.e., 50, 90, and 120 days after sowing of faba bean). During the second winter cultivation period, soil samples were collected: (a) before crop establishment, (b) after the basal dressing, (c) at the stage of flower head formation, (d) at the end of broccoli cultivation period, (e) at the 50% flowering stage of faba bean plants, and (f) after incorporation of plants residues into the soil (i.e., 0, 30, 60, 90, 120, and 140 days after crop establishment). During the summer cultivation period, soil samples were collected after application of basal dressing, at the stage of 50% flowering stage of the common bean plants, during the harvest period and at the end of the common bean cultivation period (i.e., 10, 40, 70, and 100 days after crop establishment). During the second year of the bean crop, soil samples were collected before basal dressing, after crop establishment, at the stage of 50% flowering, during the harvesting period and at the end of the common bean cultivation period (i.e., 0, 10, 40, 80, and 100 days after crop establishment).

The soil samples were oven-dried at 40 °C for at least three days until their weight stabilized to a constant level. Subsequently, the samples were sieved (2 mm diameter) and analyzed to determine the concentrations of organic nitrogen, ammonium, soil organic matter, and plant-available P and K concentrations. To identify the concentration of mineral N (NO₃-N, NH₄-N) in the soil, the soil samples were extracted with 1M KCl solution [28]. Subsequently, the NO₃-N and NH₄-N were determined in soil extracts through the Copperized Cadmium Reduction Method and Indophenol Blue method, respectively [28]. Furthermore, the same samples were also extracted through the Mehlich III
method [29] to estimate the available P by molybdate colorimetry [30] and exchangeable K using a flame photometer. Finally, the soil organic matter was also estimated through the method of Walkley Black [31].

2.3.4. Biological N Fixation and Tissue Nitrogen Concentrations

Shoot samples from faba bean and common bean plants were collected at the flowering stage to determine the biological N fixation and tissue N concentrations. The amounts of N derived from the atmosphere were estimated by applying the natural abundance method of isotope $^{15}$N. The $^{15}$N concentration in the plant samples was determined in the Stable Isotope Facility of UC-Davis, CA, USA, using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (EA-IRMS, Europa Scientific, Crewe, UK). The obtained values were used to identify the differences ($\delta^{15}$N) in the abundance of $^{15}$N in each plant tissue and in the atmospheric N, which is a known constant (0.3663% $^{15}$N), [32]. The $\delta^{15}$N values are usually expressed as $\delta^{15}$N or parts per thousand (‰) relative to the $^{15}$N content of atmospheric N$_2$ and are estimated through the following equation [33]:

$$\delta^{15}N(\%) = \frac{\text{atom}\%^{15}N\text{ sample} - 0.3663}{0.3663} \times 1000 \quad (1)$$

The proportion of N derived from atmosphere (% Ndfa) in legume was calculated using the $\delta^{15}$N values of the tested legume and a non-legume plant grown in the same soil at the same growth stage (reference plant) through the following equation [34]:

$$N\text{dfa}(\%) = \frac{\delta^{15}N\text{ of reference plant} - \delta^{15}N\text{ of legume}}{\delta^{15}N\text{ of reference plant} - B} \times 100 \quad (2)$$

where the ‘B’ value is $\delta^{15}$N (‰) of the respective legume grown on an inert medium and starved of N throughout its life, thereby being fully dependent upon N$_2$ fixation. In the current study, the B values used were (−0.5) for faba bean and (−2.16) for common bean, as suggested by Unkovich et al. [33]. The reference plant used for the determination of % Ndfa in faba bean was Ailanthus altissima sp., which was collected from faba bean plots. Reference plants of the same species were also collected from common bean plots and used for the determination of % Ndfa in the summer experiments. Samples were collected separately from farming plots treated differently during each experiment.

The total amount of N$_2$ derived from the atmosphere in the aboveground dry biomass (DM) of either faba bean or common bean (BNF, kg ha$^{-1}$) was estimated through the following equation [35]:

$$BNF = N \times DB \times N\text{dfa} ÷ 100 \quad (3)$$

where N is the total amount of N per area unit that was fixed biologically in the epigeous plant parts of the legume crop.

2.4. Statistical Analysis

In the winter cultivation period, one-way ANOVA analysis was applied to identify the main effects of the five different farming practices on soil fertility during the whole cultivation period. Furthermore, to investigate the main effects of different cultivation systems of broccoli (organic vs. conventional) and the inoculation of faba bean with rhizobia (inoculated vs. non-inoculated), a one-way ANOVA analysis was also applied. In the subsequent summer cultivation period, a two-factorial ANOVA analysis was applied to evaluate the effects of different farming practices of the preceding winter (Factor 1) and inoculation of common bean with rhizobia or not (Factor 2). The treatment means were separated by applying the Duncan’s Multiple Range Test when the two-way ANOVA analysis was significant at $p \leq 0.05$ level. The statistical analysis was conducted using the STATISTICA 12.0 software package (StatSoft Inc., Tulsa, OK, USA).
3. Results

3.1. Growth Parameters

Inoculation of faba bean plants used as green manure with Rhizobium laguerreae VFLE1 enhanced significantly the shoot fresh weight (SFW) by 58% ($F = 7.089, p = 0.037$) and 24% ($F = 8.826, p = 0.025$) during the first and the second winter cultivation period, respectively, compared to that of non-inoculated plants (Figure 3).

![Figure 3. Impact of faba bean inoculation with Rhizobium laguerreae VFLE1 on shoot fresh weight (SFW) at the 50% flowering stage during the 1st and 2nd winter cultivation period. For each cultivation period, different letters at each bar indicate significant differences according to Duncan’s multiple range test ($p < 0.05$). Vertical bars indicate standard errors of means (n = 8).](image)

In the first experimental year, the SFW of common bean plants at the 50% flowering stage was significantly higher when the preceding winter crop was conventional broccoli, compared to all other three pre-crop treatments (Table 3). Moreover, the inoculation of common bean plants with Rhizobium tropici CIAT 899 enhanced the SFW compared to non-inoculated plants. During the second cultivation period, the application of faba bean as green manure increased the SFW of common bean compared to that measured in common bean plants grown in plots with organically cultivated broccoli and fallow as pre-crop treatments. However, the SFW of common bean was similar in plots cultivated with faba bean and those conventionally cultivated with broccoli during the preceding winter. Nevertheless, the SFW of common bean was reduced by almost 50% in the second experimental year compared to the first year, regardless of the treatment applied.

During the second summer cultivation period, the inoculation of common bean with Rhizobium tropici CIAT 899 did not affect the shoot biomass production of common bean plants (Table 3). No interaction among the pre-crop treatments and the inoculation of common bean with Rhizobium tropici CIAT 899 was found.
Table 3. Impact of different pre-crop treatments during the cold season and inoculation with *Rhizobium tropici* CIAT 899 on shoot fresh weight (SFW) of the common bean at the 50% flowering stage in both experimental years.

| Pre-crop treatment     | 1st Year g plant⁻¹ | 2nd Year g plant⁻¹ |
|------------------------|---------------------|--------------------|
| Organic broccoli       | 268 b               | 142 bc             |
| Conventional broccoli  | 408 a               | 167 ab             |
| Fallow                 | 224 b               | 117 c              |
| Green manure           | 296 b               | 185 a              |

Inoculation with rhizobia

| Inoculation            | 1st Year | 2nd Year |
|------------------------|----------|----------|
| Inoculated             | 344      | 151      |
| Non-inoculated         | 253      | 154      |

| Statistical significance | 1st Year | 2nd Year |
|--------------------------|----------|----------|
| Pre-crop                 | F = 9.036, p = 0.0003 | F = 4.43, p = 0.007 |
| Inoculation              | F = 12.152, p = 0.002 | ns |
| Pre-crop x inoculation   | ns       | ns       |

Means of pre-crop treatment (n = 8) and inoculation with rhizobia (n = 16) followed by different letters within the same column indicate significant differences for both factors according to Duncan’s multiple range test, ns = not significant.

3.2. Yield Characteristics

In the first experimental year, the different farming practices applied (organic vs. conventional) had no impact on the flower head yield of broccoli. No pest or fungal diseases occurred during the first experimental year, and thus the only difference between the two systems was the origin of the N applied (Figure 4).

![Figure 4](image-url) Impact of the different farming systems (organic vs. conventional) on fresh weight (FW) of broccoli flower heads during the 1st and 2nd experimental year. For each year, different letters above each bar indicate significant differences according to the t-test (p < 0.05). Vertical bars indicate standard errors of means (n = 8).

In the following winter cultivation period, however, the lower inputs of N in the organic compared to the conventional system, and the differences in the fertilization scheme between the two systems reduced significantly by 20% (F = 26.048, p = 0.0002) the fresh weight of flower head harvested from organic broccoli compared to that obtained from the conventional plots (Figure 4).
Green manure of faba bean crop in both rotation years enhanced the pod yield of the subsequent crop of organic common bean compared to organic broccoli as preceding crop. However, the yield of the common bean obtained from the plots with faba bean cultivated as green manure was similar to that obtained when the pre-crop treatment during the winter was fallow or conventional broccoli in both experimental years. Furthermore, the number of pods per plant was restricted in organic farming of common bean established just after organic farming of broccoli compared to the other three treatments during the second experimental year, while no treatment impact was found during the 1st experimental year (Table 4). Moreover, the inoculation of common bean plants with *Rhizobium tropici* CIAT 899 did not significantly affect the total yield of fresh pods and the number of pods per plant in both experimental years. However, both the fresh pod yield of the common bean and the number of pods per plant were reduced by almost 36% in the second experimental year compared to the first year, regardless of the treatment applied. No interaction among the pre-crop treatments and the inoculation of common bean with *Rhizobium tropici* CIAT 899 was found.

**Table 4.** Impact of different pre-crop treatments during the wintertime and inoculation of the common bean with *Rhizobium tropici* CIAT 899 on total fresh weight (TFW) and number (No) of pods per plant in a summer open-field crop of the common bean during two successive experimental years.

| Yield Characteristics of Common Bean | Main Effects | | |
|-------------------------------------|--------------|--------------|-------------------|
|                                     | 1st Year     | 2nd Year     |                   |
|                                     | TFW of pods  | Pods         | TFW of pods       | Pods         |
|                                     | (t ha⁻¹)     | (No 10⁵ ha⁻¹)| (t ha⁻¹)         | (No 10⁵ ha)  |
| **Pre-crop**                        |              |              |                   |               |
| Organic broccoli                    | 42.36 b      | 45.95        | 26.70 b           | 27.74 b      |
| Conventional broccoli               | 52.84 a      | 51.96        | 33.70 a           | 34.06 a      |
| Fallow                              | 47.40 ab     | 47.15        | 29.74 ab          | 30.44 ab     |
| Green manure                        | 49.67 a      | 50.57        | 32.07 a           | 32.27 a      |
| **Inoculation with rhizobia**       |              |              |                   |               |
| Inoculated                          | 48.11        | 48.73        | 31.58             | 32.20        |
| Non-inoculated                      | 48.03        | 49.08        | 29.53             | 30.13        |
| **Statistical significance**        |              |              |                   |               |
| Pre-crop                            | *F* = 5.274  | ns           | *F* = 4.173       | *F* = 3.359  |
| Inoculation                         | *p* = 0.006  | ns           | *p* = 0.016       | *p* = 0.035  |
| Pre-crop × Inoculation              | ns           | ns           | ns                | ns           |

Means of pre-crop treatment (n = 8) and inoculation with rhizobia (n = 16 followed by different letters within the same column indicate significant differences for both factors according to Duncan’s multiple range test. *ns* = not significant.

3.3. Soil Nutrient Content

During the winter cultivation period of the first experimental year, higher amounts of soil NO₃⁻N (Figure 5A) were measured in the organic broccoli crop (*F* = 6.264, *p* = 0.002) during the formation of flower heads (i.e., 50 days after crop establishment, DACE), compared to the other three treatments applied in autumn-winter 2017–2018. However, at the end of the broccoli cultivation period (i.e., 90 DACE), the soil nitrate levels decreased significantly in plots cultivated organically with broccoli to similar levels with those found in the other treatments. The soil NO₃⁻N levels in plots cultivated with faba bean decreased 90 DACE compared to 50 DACE but 120 DACE, i.e., just before the incorporation of the plants into the soil as green manure (at the 50% flowering stage), they recovered again to similar levels as to that on 50 DACE. The soil nitrate levels did not differ significantly between the faba bean inoculated and non-inoculated with *Rhizobium laguereae* strain VFLE1 at all sampling dates, and therefore, in Figure 5A, the mean value of the two faba bean treatments is presented.
In the subsequent common bean crop, the soil NO$_3$-N concentrations were at their highest levels shortly after crop establishment (DACE), ranging from 80 to 100 mg kg$^{-1}$ dry soil, with the lowest levels found in non-cultivated (fallow) plots during the preceding winter (Figure 5B). Subsequently, the soil NO$_3$-N levels decreased in all treatments, and at the stage of 50% flowering, particularly 40 DACE, the higher levels were found in plots with organic broccoli as preceding winter crop ($F = 3.143, p = 0.044$). The soil NO$_3$-N decreased further to levels ranging from 20 to 40 mg kg$^{-1}$ dry soil during harvesting, particularly 70 DACE, with the lowest levels measured in plots with organic broccoli and fallow as pre-crop treatments during the winter ($F = 6.079, p = 0.003$). At crop termination, i.e., 100 DACE, the soil NO$_3$-N increased compared to the previous sampling date, with the lowest and highest values
found in plots left fallow and cultivated conventionally with common bean following conventional broccoli cultivation, respectively \( F = 10.05, p = 0.0002 \).

During the winter cultivation period of the second experimental year, conventional farming practices significantly increased the NO\(_3\)-N concentration in the soil after the basal dressing application \((F = 21.78, p \leq 0.000)\) and during the formation of broccoli’s flower heads \((F = 6.178, p = 0.002)\), compared to all other treatments. The lowest soil NO\(_3\)-N concentration was found in the fallow treatment. After harvesting of broccoli, the soil NO\(_3\)-N concentration was similar in all treatments, while after the incorporation of plant residues of the individual crops into the soil, significantly \((F = 3.44, p = 0.03)\) higher levels of soil NO\(_3\)-N concentration were found in the plots accommodating faba bean as green manure (Figure 5C). The incorporation of plant residues in all plots of the winter crops enhanced the N availability in the soil before the establishment of the subsequent common bean crop (Figure 5C).

Ten days after the establishment of the common bean crop in the second experimental year, the soil NO\(_3\)-N concentration was highest \((F = 4.592, p = 0.011)\) in the conventional common bean crop followed by the organic common bean grown after the application of faba bean as green manure (Figure 5D). At the 50% flowering stage, the plots of common bean established after the winter fallow resulted in significantly \((F = 3.206, p = 0.041)\) lower soil NO\(_3\)-N concentrations compared to the other three treatments, which were similar. In the next sampling date, i.e., 80 DACE, the highest soil NO\(_3\)-N concentrations (about 100 mg kg\(^{-1}\)) were measured in organic plots treated with green manure during the preceding winter and plots with conventional common bean, whereas the corresponding values in the plots with organic broccoli and fallow during the preceding winter were much lower (58 and 40 mg kg\(^{-1}\), respectively) \((F = 7.121, p = 0.001)\). Finally, at crop termination, the application of green manure resulted in appreciably higher soil NO\(_3\)-N levels than in the other three treatments, while the plots with organic broccoli and fallow during the preceding winter rendered significantly \((F = 12.533, p = 0.00004)\) lower yield also in comparison with the conventional common bean.

During the winter cultivation period of the first year of the rotation scheme, higher amounts of soil NH\(_4\)-N (Figure 6A) were recorded in the organic broccoli during the formation of broccoli flower heads (i.e., 50 DACE) compared to the other three treatments \((F = 4.238, p = 0.013)\). No significant differences were found among the other three treatments. Furthermore, no significant increase in the NH\(_4\)-N levels in the faba bean crop was found during the 50% flowering stage (i.e., 120 days DACE) compared to all other sampling dates. In the subsequent common bean crop, the soil NH\(_4\)-N levels ranged from 6 to 9 mg kg\(^{-1}\) during the cropping period, without significant differences between treatments (Figure 6B).

During the autumn-winter cultivation period of the second experimental year, the soil NH\(_4\)-N level decreased from 18–20 mg kg\(^{-1}\) on the day of crop establishment to about 11–12 mg kg\(^{-1}\) 30 DACE, without significant differences between treatments (Figure 6C). At the stage of the flower heads formation (i.e., 60 DACE), the soil NH\(_4\)-N concentration increased significantly \((F = 7.128, p = 0.001)\) in the plots with organic broccoli compared to the other three treatments but in the subsequent sampling dates it was reduced to similar levels with all other treatments. At the 2nd spring-summer cultivation period, the soil NH\(_4\)-N levels increased up to the stage of 50% flowering (i.e., 40 DACE). After this stage, the NH\(_4\)-N levels decreased up to the end of the experiment, while no significant differences were recorded among the different treatments (Figure 6D).

The contribution of rhizobia inoculation to soil N availability did not differ significantly between the inoculated and non-inoculate faba bean during the autumn-winter cultivation period in both experimental years. In addition, in both experimental years, the incorporation of either inoculated or non-inoculated faba bean as green manure did not influence the N availability of the subsequent common bean crop. Moreover, inoculation of common bean with the strain *Rhizobium tropici* CIAT 899 did not enhance the N availability during the summer cultivation periods. Therefore, as the inoculation of legume crops with rhizobia in both experimental years had no significant impact on soil N availability, the data are not presented.
Figure 6. Impact of pre-crop treatments during the wintertime on NH$_4$-N concentration in the soil during the two experimental years. Vertical bars indicate standard errors of means (n = 8), while * indicates significant differences according to Duncan’s multiple range test (p < 0.05), significant at p < 0.05. (A) Soil NH$_4$-N concentrations in the different treatments applied as preceding crops during autumn-winter 2017–2018, particularly at 50, 90, and 120 days after crop establishment (DACE). (B) Soil NH$_4$-N concentrations after crop establishment, at the 50% flowering stage, during harvest, and at crop termination (10, 40, 70, and 100 DACE) in the common bean crop during spring-summer 2018, as influenced by the preceding winter crop. (C) Soil NH$_4$-N concentrations before basal dressing, after crop establishment, at the stage of broccoli flower head formation, at crop termination of broccoli, at 50% flowering of faba bean, and after incorporation of plant residues into the soil (0, 30, 60, 90, 120, and 140 DACE) in the different treatments applied as preceding crops during autumn-winter 2018–2019. (D) Soil NH$_4$-N concentrations before and after the establishment of common bean crop, at the 50% flowering stage, at harvest, and at crop termination (0, 10, 40, 80, and 100 DACE, respectively) during spring-summer 2019, as influenced by the preceding winter crop.

The soil P concentrations in the autumn-winter crops during the first experimental year decreased slightly with time without significant differences between treatments (Figure 7A). In the subsequent common bean crop, the soil P concentrations increased slightly at the 50% flowering stage (i.e., 40 DACE) without any significant differences between treatments (Figure 7B). However, during the harvesting period, the soil P tended to decrease in all treatments without any significant differences between them (Figure 7B).
Figure 7. Impact of pre-crop treatments during the wintertime on P concentration in the soil during the two experimental years. Vertical bars indicate standard errors of means (n = 8), while * and ** indicate significant differences according to Duncan’s multiple range test (p < 0.05), significant at p < 0.05 and p < 0.01, respectively. (A) Soil P concentrations in the different treatments applied as preceding crops during autumn-winter 2017–2018, particularly at 50, 90, and 120 days after crop establishment (DACE). (B) Soil P concentrations after crop establishment, at the 50% flowering stage, during harvest, and at crop termination (10, 40, 70, and 100 DACE) in the common bean crop during spring-summer 2018, as influenced by the preceding winter crop. (C) Soil P concentrations before basal dressing, after crop establishment, at the stage of broccoli flower head formation, at crop termination of broccoli, at 50% flowering of faba bean, and after incorporation of plant residues into the soil (0, 30, 60, 90, 120, and 140 DACE) in the different treatments applied as preceding crops during autumn-winter 2018–2019. (D) Soil P concentrations before and after the establishment of common bean crop, at the 50% flowering stage, at harvest, and at crop termination (0, 10, 40, 80, and 100 DACE, respectively) during spring-summer 2019, as influenced by the preceding winter crop.

During autumn-winter of the 2nd experimental year, significant lower soil P concentrations were recorded in non-cultivated plots (fallow) after the establishment of the basal dressing (F = 4.413, p = 0.012) and during the formation of broccoli flower heads (F = 6.585, p = 0.002) (i.e., 30 and 60 DACE, respectively), compared to all other treatments (Figure 7C). However, thereafter the soil P levels tended to decrease in all treatments, without significant differences between them. In the subsequent common bean crop during the 50% flowering stage (F = 3.202, p = 0.041) and harvest period (F = 4.334, p = 0.014), the soil P concentrations were higher in the plots with organic broccoli during the winter,
while the lowest levels were measured when common bean was organically cultivated in plots not cultivated during the winter (Figure 7D).

In both autumn-winter cultivation periods, the inoculation of faba bean with *Rhizobium laguerreae VFLE1* had no significant impact on soil P concentration. During the spring-summer cultivation periods, the incorporation of inoculated or non-inoculated green manure into the soil did not affect the P concentration in the soil of the subsequent organic common bean crop, while no variations were also observed in the soil P concentration of common bean either inoculated with *Rhizobium tropici CIAT 899* or non-inoculated, irrespective of the farming practices. Therefore, as the inoculation of legume crops with rhizobia in both experimental years did not influence the soil P availability, the data are not presented.

The soil K concentration was not influenced by different farming practices or by the inoculation of legumes with rhizobia in both experimental years and cultivation periods. Therefore, no data on the soil K levels are shown.

### 3.4. Soil Organic Matter

According to Table 1, the level of soil organic matter (SOM) in the experimental field was already high before the establishment of the field experiment. During the 1st winter cultivation period, the different farming practices did not affect the SOM (Figure 8A). During the subsequent common bean crop, the conventional farming practices tended to decrease the SOM levels during the harvesting period compared to all treatments with organic bean cropping, but the difference disappeared at crop termination (Figure 8B).

During the second winter cultivation period, the lowest SOM levels were detected in the plots cultivated with conventional broccoli after the establishment of basal dressing (\(F = 3.033, p = 0.046\)) and broccoli cultivation period (\(F = 3.09, p = 0.043\)). Besides, after the incorporation of plant residues, significantly (\(F = 4.704, p = 0.009\)) higher amounts of SOM were recorded in the plots that accommodated organic farming of broccoli and green manure crops (Figure 8C). In the subsequent common bean crop, significantly lower levels of SOM were measured in plots with common bean following conventional cultivation of broccoli during the preceding winter (before crop establishment: \(F = 3.349, p = 0.035\)/after the basal dressing: \(F = 5.116, p = 0.007\)/50% flowering stage: \(F = 5.805, p = 0.004\)/during the harvest period: \(F = 4.204, p = 0.016\)) (Figure 8D).

Finally, the SOM levels were not affected by the inoculation of faba bean or common bean with rhizobia during both experimental years. Therefore, the data from both inoculated and non-inoculated plots were pooled together in Figure 8.
Figure 8. Impact of pre-crop treatments during the wintertime on soil organic matter (SOM) during the two experimental years. Vertical bars indicate standard errors of means (n = 8), while * and ** indicate significant differences according to Duncan’s multiple range test (p < 0.05), significant at p < 0.05 and p < 0.01, respectively. (A) SOM in the different treatments applied as preceding crops during autumn-winter 2017–2018, particularly at 50, 90, and 120 days after crop establishment (DACE). (B) SOM after crop establishment, at the 50% flowering stage, during harvest, and at crop termination (10, 40, 70, and 100 DACE) in the common bean crop during spring-summer 2018 as influenced by the preceding winter crop. (C) SOM before basal dressing, after crop establishment, at the stage of broccoli flower head formation, at crop termination of broccoli, at 50% flowering of faba bean, and after incorporation of plant residues into the soil (0, 30, 60, 90, 120, and 140 DACE) in the different treatments applied as preceding crops during autumn-winter 2018–2019. (D) SOM before and after the establishment of common bean crop, at the 50% flowering stage, at harvest, and at crop termination (0, 10, 40, 80, and 100 DACE, respectively) during spring-summer 2019, as influenced by the preceding winter crop.

3.5. Nitrogen Content and BNF Activity of Legumes

During both winter cultivation periods, the inoculation of the faba bean with Rhizobium laguerreae VFLE1 did not affect the shoot N concentration and C/N ratio. On the other hand, inoculation with rhizobia increased the percentage of N derived from the atmosphere (% Ndфа) in both experimental years by 13% and 9%, respectively (Table 5). The increased % of Ndфа in the inoculated plants, in combination with the increased dry biomass, resulted in higher amounts of fixed N by 68% in the
1st and 52% in 2nd autumn-winter cultivation period. Moreover, the N concentration, dry biomass, and BNF in the 2nd cultivation period was higher compared to the 1st cultivation period.

Table 5. Impact of faba bean inoculation with *Rhizobium laguerreae VFL1* on the C/N ratio, N concentration (%), %Ndfa, dry biomass (t ha\(^{-1}\)), and amount of biologically fixed N (BNF, kg ha\(^{-1}\)) in the aboveground plant biomass, in both experimental years.

| Treatment                      | C/N   | N (%) | Ndfa (%) | Dry Biomass (t ha\(^{-1}\)) | BNF (kg ha\(^{-1}\)) |
|--------------------------------|-------|-------|----------|-----------------------------|---------------------|
| 1st Experimental year (2017–2018) |       |       |          |                             |                     |
| Inoculated                     | 11.43 | 3.66  | 86.00    | 8.08                        | 255                 |
| Non-inoculated                 | 10.36 | 3.93  | 75.9     | 5.09                        | 152                 |
| Statistical significance       | ns    | ns    |          |                             |                     |
| F = 23.474                     |       |       |          |                             |                     |
| F = 9.279                      |       |       |          |                             |                     |
| F = 7.7454                     |       |       |          |                             |                     |
| p = 0.002                      |       |       |          |                             |                     |
| p = 0.022                      |       |       |          |                             |                     |
| p = 0.032                      |       |       |          |                             |                     |
| 2nd Experimental year (2018–2019) |       |       |          |                             |                     |
| Inoculated                     | 9.58  | 4.7   | 76.3     | 21.79                       | 780                 |
| Non-inoculated                 | 8.65  | 4.2   | 69.7     | 17.51                       | 514                 |
| Statistical significance       | ns    | ns    |          |                             |                     |
| F = 21.43                      |       |       |          |                             |                     |
| F = 7.7481                     |       |       |          |                             |                     |
| F = 27.549                     |       |       |          |                             |                     |
| p = 0.004                      |       |       |          |                             |                     |
| p = 0.032                      |       |       |          |                             |                     |
| p = 0.002                      |       |       |          |                             |                     |

Means of inoculation with rhizobia (n = 4). ns= non-significant.

In the 1st spring-summer cultivation period, the C/N ratio and N concentration in the aboveground biomass of common bean plants were not affected by the different crops during the preceding winter or by the inoculation of common bean with *Rhizobium tropici CIAT 899* (Table 6). The highest percentage of N derived from the atmosphere (%Ndfa) was found in plants cultivated conventionally or in those cultivated organically following the green manure application of faba bean, whereas the lower %Ndfa was recorded in the common bean plants cultivated in the plots left fallow during the preceding winter. Moreover, the inoculation of common bean with rhizobia significantly increased the %Ndfa, irrespective of the treatment applied during the preceding winter. Besides, conventional farming practices increased the dry biomass of the common bean crop compared to all organic treatments. The BNF increased in common bean plants cultivated in plots with conventional broccoli or green manure during the winter, while in those left fallow during the preceding winter the amount of biologically fixed N by organic common bean was the lowest. Finally, inoculation with rhizobia enhanced the amount of biologically fixed N when compared to the non-inoculated plants, (Table 6). No interaction between the applied farming practices and the inoculation of common bean with *Rhizobium tropici CIAT 899* was recorded.

In the 2nd summer cultivation period, the C/N ratio and the N concentration in the aboveground biomass of common bean plants were not affected by the different treatments during the preceding winter or by inoculation with *Rhizobium tropici CIAT 899* (Table 7). In addition, the application of green manure during the preceding winter restricted the %Ndfa by the common bean. The highest %Ndfa was recorded in the plots with organic farming of common bean following fallow or organic broccoli during the preceding winter. Furthermore, the higher N availability in green manure treatment enhanced the dry biomass of organically cultivated common bean, while the limited N availability in organic farming of common bean after fallow and organic broccoli restricted the dry biomass of the common bean plants (Table 7). Therefore, this inverse relation between %Ndfa and dry biomass of common bean plants due to the variance of N availability resulted in similar total amounts of N fixed biologically by common bean in the treatments with different farming practices. The inoculation of common bean with rhizobia had no significant impact on the %Ndfa, dry biomass, and BNF (Table 7). Finally, no interaction between the winter treatments and the inoculation of common bean with *Rhizobium tropici CIAT 899* was found.
Table 6. Impact of the different pre-crop treatment and inoculation of common bean with *Rhizobium tropici* CIAT 899 on C/N ratio, N content (%), %Ndfa, dry biomass (t ha⁻¹), and amount of biological fixed N (BNF, kg ha⁻¹) of common bean cultivated during spring-summer 2018.

| C/N   | N (%) | Ndfa (%) | Dry biomass (t ha⁻¹) | BNF (kg ha⁻¹) |
|-------|-------|----------|----------------------|---------------|
| Pre-crop |       |          |                      |               |
| Organic broccoli | 10.99 | 3.62     | 12.61 ab             | 2.49 b        |
| Conventional broccoli | 10.61 | 3.77     | 18.53 a              | 3.46 a        |
| Fallow | 11.86 | 3.37     | 9.61 b               | 2.08 b        |
| Green Manure | 10.63 | 3.77     | 18.47 a              | 2.40 b        |

Inoculation with rhizobia

| C/N   | N (%) | Ndfa (%) | Dry biomass (t ha⁻¹) | BNF (kg ha⁻¹) |
|-------|-------|----------|----------------------|---------------|
| Inoculated | 10.64 | 3.73     | 16.99                | 2.80          |
| Non-inoculated | 11.41 | 3.54     | 12.62                | 2.42          |

Statistical significance

| C/N   | N (%) | Ndfa (%) | Dry biomass (t ha⁻¹) | BNF (kg ha⁻¹) |
|-------|-------|----------|----------------------|---------------|
| Pre-crop |       |          |                      |               |
| Inoculation |       |          |                      |               |
| Pre-crop × Inoculation |       |          |                      |               |

Means of pre-crop treatment (n = 8) and inoculation with rhizobia (n = 16) followed by different letters within the same column indicate significant differences for both factors, according to Duncan’s multiple range test. ns = not significant.

Table 7. Impact of the different pre-crop treatment and inoculation of common bean with *Rhizobium tropici* CIAT 899 on C/N ratio, N concentration (%), %Ndfa, dry biomass (t ha⁻¹), and amount of biologically fixed N (BNF, kg ha⁻¹) of common bean cultivated during spring-summer 2019.

| C/N   | N (%) | Ndfa (%) | Dry Biomass (t ha⁻¹) | BNF (kg ha⁻¹) |
|-------|-------|----------|----------------------|---------------|
| Pre-crop |       |          |                      |               |
| Organic broccoli | 14.41 | 2.78     | 34.94 a              | 1.41 bc       |
| Conventional broccoli | 13.99 | 2.90     | 28.63 ab             | 1.65 ab       |
| Fallow | 14.36 | 2.81     | 40.69 a              | 1.20 c        |
| Green manure | 13.83 | 2.90     | 21.18 b              | 1.82 a        |

Inoculation with rhizobia

| C/N   | N (%) | Ndfa (%) | Dry Biomass (t ha⁻¹) | BNF (kg ha⁻¹) |
|-------|-------|----------|----------------------|---------------|
| Inoculated | 14.25 | 2.82     | 32.28                | 1.56          |
| Non-inoculated | 14.05 | 2.87     | 30.44                | 1.48          |

Statistical significance

| C/N   | N (%) | Ndfa (%) | Dry biomass (t ha⁻¹) | BNF (kg ha⁻¹) |
|-------|-------|----------|----------------------|---------------|
| Pre-crop |       |          |                      |               |
| Inoculation |       |          |                      |               |
| Pre-crop × Inoculation |       |          |                      |               |

Means of pre-crop treatment (n = 8) and inoculation with rhizobia (n = 16) followed by different letters within the same column indicate significant differences for both factors, according to Duncan’s multiple range test. ns = not significant.

4. Discussion

According to Berry et al. [36], the gap in yield between conventional and organic cultivation systems is mainly affected by the timing of N availability in soil and not by the total N inputs in both systems. Other factors affecting the yield gap are the type of crop, the environmental conditions, and the variability of organic and conventional farming practices worldwide [37]. As the mineral N inputs and management of weeds and pests using man-made agritoxins are prohibited in organic agriculture,
crop rotation is considered an alternative approach to improve N supply and further soil structure and fertility and contribute to fewer weeds and pest pressure [38]. In addition, Bullock et al. [38] reported that the beneficial effects of crop rotation are best achieved under more diverse crop-sequences and over the long-term. Consequently, the accumulation of system-function benefits that can provide sufficiently high yields requires time in organic crops. In particular, the gap yield between organic and conventional farming systems is larger during the first years of conversion from conventional to organic farming and tends to decrease in crops managed organically for three or more years [10]. On the other hand, Martini et al. [39] claimed that high yield is feasible during the transition from conventional to organic agriculture under adept organic management.

In the present study, the applied organic farming practices did not restrict the yield of broccoli during the first winter cultivation period. This could be ascribed to the high nutrient reserves in the field before the establishment of the two-year crop rotation scheme, including the high soil organic matter content. The high level of soil organic matter is attributed to the use of an experimental field that remained uncultivated during the last five years before establishing the current research, and the weeds were incorporated into the soil during the initial soil cultivation. However, during the following winter cultivation period, the organic farming practices restricted the N availability in soil, resulting in reduced yield of organically cultivated broccoli by 20%. A similar reduction in broccoli yield under organic farming practices was also recorded by Yildirim et al. [40], who found that the yield of organic broccoli was 23% lower compared to that found in a conventional broccoli crop. According to the study of Øvsthus et al. [41], where broccoli was cultivated either organically using sheep manure as an N source, or conventionally following a two-year crop rotation scheme, crop yield was restricted by the organic farming practices in both cultivation periods, even though N inputs were the same in both systems. However, in the above study, all N inputs to the organic crop were provided as basal dressing, and this might have restricted N availability at critical developmental stages of the crop.

In the current study, the restricted N availability in the soil was presumably the main factor limiting the production of organically common bean crop cultivated after organic broccoli. This is in agreement with the study of Kontopoulou et al. [42], in which the yield of the cultivated common bean was reduced by 33% under organic farming practices due to limited N availability in the soil. On the other hand, organic farming practices did not restrict the yield of common bean cultivated after green manure application, which is in agreement with several studies [15,16,43,44]. Application of faba bean as green manure is also beneficial in cereal crops. Indeed, in another study [45], the inclusion of faba bean as green manure to a long-term rotation scheme with cereals resulted in increased yield compared to conventional cereal monoculture.

The beneficial effect of inoculating faba bean with *Rhizobium laguerreae VFLE1* on plant growth found in this study are in agreement with previous reports [46]. Furthermore, common bean growth was also enhanced by the inoculation of common bean with rhizobia in the current study. However, a beneficial effect of inoculating common bean with rhizobia on plant biomass production was recorded only in the first year of the crop rotation scheme. This could be ascribed to the spreading of the above inoculant across the experimental field after its application during the first experimental year, in agreement with previous findings in another study in which cowpea was applied as green manure in an organic tomato cultivation [47].

Unlike common bean, the faba bean benefited from inoculation with *Rhizobium laguerreae VFLE1* in terms of biological N fixation in both winter cultivation periods, as shown in Table 5. This finding is in agreement with a study of Hungria et al. [48], who claimed that re-inoculation of soybean each year enhanced its N-fixing activity despite the sufficient population of effective rhizobia in the soil. Besides, according to Herridge et al. [18], a lower population of effective inoculants in the soil could restrict the N fixation of the host legume. The different effects of re-inoculating faba bean and soybean with rhizobia, compared to bean and cowpea, show that a benefit from rhizobia re-inoculation should not be always anticipated as the efficiency of this treatment may also depend on factors such as the legume species, the soil conditions, climatic parameters, etc. On the other hand, contrary to the
studies of Bard El-in et al. [46] and Denton et al. [17], the inoculation of faba bean with rhizobia in the current study did not enhance the N (%) content in the plant shoot. Nevertheless, due to the higher shoot biomass inoculation of faba bean finally provided a net gain in the total amount of biologically fixed N\textsubscript{2} per cultivated area unit. The values of the %N\textsubscript{didas} recorded for faba bean in this study, irrespective of inoculation with rhizobia or not, are in agreement with the values recorded in the study of Jensen et al. [15], where the %N\textsubscript{didas} of faba bean ranged from 58% to 88% when 30–50 kg N ha\textsuperscript{-1} were supplied as basal dressing.

The cultivation of faba bean as green manure during both experimental years enhanced the N availability in the subsequent common bean crop, in agreement with previous relevant reports [15,16,43]. However, during the second experimental year, faba bean provided higher amounts of available N to the subsequent crop compared to the first experimental year as indicated in the comparison of panels B and D in Figure 5. This was partially ascribed to the higher N (%) content and the lower C/N ratio in the faba bean residues incorporated into the soil in the second experimental year. However, it was also ascribed to the higher biomass of faba bean incorporated into the soil in the second year. High N concentrations and low C/N ratios in plant residues incorporated into the soil enhance the microbial activity, thereby increasing their decomposition rates, and providing a higher net N mineralization [49,50]. The lower N availability after the green manure application in the first year could also be attributed to the immobilization of N originating from the mineralization of green and animal manure in the microbial biomass, which is commonly observed if the soil mineral N is low [16]. An additional reason is that the N release during spring-summer of the second experimental year was also favored by green manure application during winter of the first year, as it is well known that the beneficial effects of green manure appear in the long term [51].

In the present study, the inoculated faba bean crop fixed 103 and 266 kg ha\textsuperscript{-1} more N than the non-inoculated faba bean crop during the first and second winter cultivation periods, respectively. However, the higher amounts of symbiotically fixed N did not increase the N availability and concomitantly the yield in the subsequent common bean crop compared to that obtained from plots in which the green manure resulted from non-inoculated faba bean plants. Peoples et al. [16] found that only a small percentage (11–17%) of N originating from faba bean residues was utilized by the subsequent cereal crop. Considering this, we can assume that in the present study, the inoculation of faba bean with rhizobia did not provide significantly higher amounts of N to the subsequent common bean crop that might effectively increase growth and yield. On the other hand, inoculation of faba bean with rhizobia had no impact on the tissue N concentration and C/N ratio, and thus the net mineralization of the inoculated and non-inoculated faba bean residues was similar. Since the decomposition rates in both treatments were similar, the larger amount of N resulting from the incorporation of the inoculated faba bean plants into the soil could not be available to the subsequent common bean crop in such a short period.

Green manure application increased the yield of the subsequent organic common bean crop by 17% and 20% during the 1st and 2nd year of crop rotation, respectively, compared to the yield of the organic common bean cultivated after organic broccoli. However, in the short term, the increase of common bean yield to this level is not sufficient to offset the benefit obtained from the production of another crop in the same year, particularly broccoli in the current study. Nevertheless, in the long term, the rotation of common bean during the warm season with a winter legume used as green manure in the same year is essential to maintain soil fertility in organic cropping systems. An alternative approach might be the cultivation of faba bean for fresh pod production, which allows for early harvesting and the incorporation of the crop residues into the soil after harvest, which would improve the N availability for the subsequent crop [43]. On the other hand, Patrignin et al. [52] claimed that during seed maturity, faba bean crop requires higher amounts of N compared to those fixed from the atmosphere, and thus significant amounts of N are removed from the field during the harvest.

The inoculation with rhizobia also slightly improved the biological N fixation of common bean crop during the first summer cultivation period, while this benefit was not recorded during the second
experimental year when the common bean crop was re-inoculated. This result is in disagreement with the beneficial effect of re-inoculating faba bean with *Rhizobium laguerreae* VFLE1 on BNF activity. According to Hungria et al. [53], the re-inoculation of common bean crop with *Rhizobium tropici* CIAT 899 enhanced nodule occupancy, even if the population of the same strain in the soil was already high due to a prior inoculation during the preceding cultivation period. However, according to the same study, the re-inoculation of common bean with the above strain had no beneficial consequences to the nodulation of the plants when the experiment was conducted under high temperature and dry climatic conditions. This statement is in agreement with the present study, where the re-inoculation of common bean with *Rhizobium tropici* CIAT 899 did not benefit the BNF activity of common bean plants during the second cultivation period when the crop was exposed to significantly higher temperatures compared to those recorded during the first experimental year. Furthermore, the BNF activity of common bean plants was affected by the N availability in the soil during the 50% flowering stage. Indeed, in both experimental years, the high N availability in the soil at crop establishment restricted the BNF efficiency of common bean. These responses of the BNF activity to N availability in the soil are in agreement with several studies [15–17,26,43,52,54], in which higher amounts of available N into the soil is considered to limit the BNF activity of legume crops.

During summer 2019, the growth and yield of common bean were significantly lower, and the BNF activity was significantly higher in all treatments compared to those found in the first experimental year in 2018, although the N availability in the soil was higher. Furthermore, the availability of P and in the soil cultivated with common bean was similar during both experimental years. This result indicates that N availability is not the only factor that affects crop performance when common bean follows common bean for a second year in the same field. The longer duration of the second winter cultivation period, which delayed the establishment of the subsequent summer cultivation period, resulted in higher mean max temperatures and dry conditions during harvesting according to the presented climate data in Figure 2, which reduced plant growth and yield. According to Agtunong et al. [55], heat stress (34 °C) during and after flowering reduced seed and pod production of common bean plants. In addition, DiPaola et al. [56] reported that high levels of available N in the soil reduced heat resistance of plants, while Bassirirad et al. [57] showed that increased soil temperatures and elevated N fertigation restricted the N uptake of common bean plants.

As the plant residues in both conventional and organic plots were incorporated into the soil, the increased SOM in those treated according to organic farming practices in the second experimental year compared to those treated conventionally is ascribed to the application of manure in the former. According to Bullock [38], the incorporation of crop residues in short-term rotation schemes does not improve the soil organic matter content to similar levels with the application of animal manure. Previous research has shown that the application of farmyard manure can also increase the soil P levels [58]. Hence, the elevated P availability in the soil when the preceding crop was organic broccoli compared to non-cultivated plots (fallow treatment) during the second cultivation year could be ascribed to the application of animal manure during the winter cultivation periods.

5. Conclusions

The yield of common bean cultivated organically in the open field during spring-summer, following organic broccoli during the preceding autumn-winter, decreased significantly due to restricted soil mineral N levels compared to conventional bean following conventional broccoli. However, the cultivation of common bean during the warm season, following a legume crop applied as green manure during the preceding winter, resulted in similar or even higher soil mineral N levels and similar yield with those found in the conventional bean crop. The lowest mineral N levels during summer in the 2nd experimental year were recorded in the fallow plots, although the yield obtained from those plots was not significantly reduced compared to that obtained from plots cultivated organically or conventionally during the winter. Unlike common bean, which did not benefit from inoculation with *Rhizobium tropici* CIAT 899, faba bean exhibited a considerable increase in plant
biomass and the total amount of biologically fixed N\textsubscript{2} per cultivated area unit after inoculation with \textit{Rhizobium laguerreae} VFLE1 in both winter cultivation periods. The yield obtained from the bean crop was substantially lower during the 2nd compared to the 1st experimental year in all treatments because, in the 1st year, the favorable weather conditions in spring allowed for earlier crop establishment. As a result, the harvesting period, which lasted 50 days in both years, started earlier in the 1st year, thereby resulting in less heat stress associated with the Mediterranean summer, and concomitantly in more vigorous plant growth and better fruit setting.

**Author Contributions:** D.S., I.K. and G.N. conceived and designed the experiments. A.T. designed and prepared rhizobial inoculations. I.K., G.N., T.N. and I.V. performed the experiments and the analyses. I.K., G.N. and D.S. wrote the paper. I.K., G.N., T.N., A.T., P.P.M.I. and D.S. reviewed the paper. All authors have read and approved the manuscript.

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