Highlights

- Higher yield and N_2-fixation in conventional than organic faba bean
- Higher yield stability in organic than conventional faba bean
- Organic faba bean yield was as or more stable than organic spring cereals
- N derived from atmosphere range was 78% to 93% and 199 to 399 kg N ha^{-1} aboveground
- N_2-fixation was underestimated with standard values, disregarding management effect

Faba bean productivity, yield stability and N_2-fixation in long-term organic and conventional crop rotations

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Abstract

Increasing the production of grain legumes in Europe will contribute to protein self-sufficiency and provide direct and indirect environmental benefits, e.g., delivering ecosystem services such as N input via biological N_2 fixation (BNF). Faba bean (*Vicia faba* L.) is the main grain legume cultivated in Europe with increasing interest from the organic sector. Agronomic and economic obstacles exist to the inclusion of grain legumes in cropping systems but could be counterbalanced by accounting for the provision of ecosystem services. Thus, variations in productivity and BNF under different management need to be investigated. We assessed productivity, yield stability and BNF in a common faba bean variety (Boxer), grown for four years (2015-2018) in a long-term crop rotation field experiment at Foulum, Denmark. We compared conventional and organic cropping systems with spring cereals and faba bean, with and without long-term use of animal manure and cover crops. N derived from atmosphere (%Ndfa), determined with the ^{15}N isotope dilution method, varied from 78% to 93% with significant effects of year and cropping system. Conventional treatments had the highest %Ndfa and yield, but the lowest yield stability. Organic treatments had problems with pests and diseases, mainly towards the end of the growing season. Quantity of BNF (qBNF) in aboveground biomass was on average 255 kg N ha^{-1} in the organic and 334 kg N ha^{-1} in the conventional systems, which would have been underestimated by up to 50 and 100 kg N ha^{-1} respectively using standard literature %Ndfa values. A correct estimation of N input via BNF has economic and environmental implications (e.g., fertilization of following crops, N losses); thus, we recommend to account for the effect of management on %Ndfa and qBNF.

Keywords: grain legume; pulses; biological N fixation; plant protein

1. Introduction

Grain legumes represent an important source of plant protein for food and feed. In 2020, grain legumes were grown on approximately 7% of total arable land worldwide (FAOSTAT, 2022) and only 2% in Europe (EUROSTAT, 2022). Europe is currently importing more than 70% of high-protein (30-50% protein content) livestock feed, such as soybean and soybean meal (European Commission, 2021). Most of the soybean imported in Europe is produced in South America, where soybean production is associated with deforestation (Gasparri et al., 2013), negative ecosystem impacts (Lathuillière et al., 2017) and large reductions in ecosystem carbon (C) stocks (Bonini et al., 2018). To deal with this, EU's Farm to Fork Strategy (European Commission, 2020) aims at increasing domestic protein feed production to reduce land demand in areas prone to deforestation. In addition, as pointed out by the EAT–Lancet Commission on healthy diets from sustainable food systems, a shift towards direct human consumption of plant protein is needed for both human and planet health (Willet et al., 2019).

Despite the European production of grain legumes still being low, it has been increasing recently, partly from the increasing demand for local and organically produced food (European Commission, 2018). However, most European countries are still far from exploiting their production potential. In Northern European countries, environmental conditions and the short growing period are not well suited for the cultivation of several grain legumes. Species like faba bean and pea are well adapted, although breeding (i.e., genetic improvement) has been limited so far. The poor adoption of grain legumes from European farmers has several reasons, including the comparative advantage in the production of cereals through the availability of nitrogen (N) fertilizers and pesticides, genetic improvement, and market dynamics (Magrini et al., 2016; Watson et al., 2017). .

Among grain legumes species, faba bean (*Vicia faba* L.) occupied 21% of the agricultural land cultivated with grain legumes in Europe in 2020 and was the main grain legume crop produced in e.g., Denmark, Italy, and the UK (EUROSTAT, 2022). Different faba bean varieties are adapted to grow over a wide range of environments, including areas with cool climate and a short growing season, such as northern Europe (Smykal et al., 2015). Grain yield of faba bean grown in long-term experiments in the UK, Sweden, and Germany ranged from 2.1 to 3.0 Mg ha^{-1}, which was on average 50% lower than that of cereals (Reckling et al., 2018). However, when grain legumes were compared to spring cereals, differences in productivity were less pronounced and yield stability was comparable (Reckling et al., 2018). This should be accounted for when assessing the performance of grain legumes, with the need to fill the current knowledge gap on the effect of management on faba bean productivity and ecosystem services. Within organic cropping systems, the lack of pest and disease management options challenge yields (Shah et al., 2017), while environmental constraints (e.g., water availability) could limit conventional production as well. Comparative studies are lacking on the productivity of faba bean under organic and conventional management with varying environmental conditions, in comparison with common spring cereals.

Besides the need to increase the production of plant protein in Europe, grain legumes provide several supporting services when included in crop rotations, such as the "break-crop" effect and N input via biological N_2 fixation (BNF) (Zhao et al., 2022). This reduces the need for N fertilizers and increases the overall fertilizer N use efficiency of the cropping system, also thanks to the potential increase in the yield of following crops (Preissel et al., 2015). Accounting for the ecosystem services provided by grain legumes could promote their cultivation (Magrini et al., 2016), and this requires assessing how management and environmental conditions can impact their effect.

The quantity of N fixed via BNF (qBNF) is crucial to determine N services provided by grain legumes, and can vary greatly due to species, cultivar, environment, and climatic conditions (Evans et al., 2001). As qBNF is a function of N yield and of the percentage of N derived from the atmosphere (%Ndfa), both these factors influence the field N balance and the N services provided by grain legumes (Evans et al., 2001). Although it is common to adopt standard mean values (e.g., Iannetta et al., 2016), %Ndfa can vary greatly, for example due to high soil N availability reducing N_2 fixation rate (e.g., van Zwieten et al., 2015). This can occur in organic cropping systems where input of animal manure and use of cover crops can build up the soil N pool, while the same would not be expected in conventional systems. Knowledge is currently lacking on how management and environmental conditions can affect %Ndfa and qBNF in grain legumes. Thus, to better assess rotational effects of grain legumes, allowing farmers to adjust N inputs to the following crop and optimize ecosystem services and economic benefits, variations in %Ndfa and qBNF need to be further investigated and quantified.

Here, we investigated how productivity, yield stability and biological N_2 fixation of faba bean varied in conventional and organic cropping systems, with and without long-term use of animal manure and cover crops. The study was conducted in Denmark for four years (2015, 2016, 2017, 2018), and faba bean was grown in rotation with spring cereals, used as means of comparison for productivity and yield stability. We hypothesized that productivity of faba bean would be lower in organic compared to conventional cropping systems, mostly due to lack of pest and disease management, and that yield stability of faba bean would be similar to that of spring cereals grown within the same cropping system (hypothesis 1). In addition, we hypothesized that %Ndfa in faba bean would be lower in treatments with long-term use of animal manure and cover crops, due to the higher availability of soil N (hypothesis 2).

2. Material and methods

2.1. Field site

This study was based on data from the fifth cycle (2015-2018) of a long-term crop rotation experiment started in 1997 at Foulum (56º 30´ N, 9º 34´ E), Denmark, to study how different cropping systems and management would affect productivity and environmental impacts on the long term (Olesen *et al.*, 2000). The climate at the study site is temperate oceanic (Cfb in the Köppen classification), with an average annual temperature of 8.6, 8.6, 8.5 and 9.2°C and average cumulative precipitation of 846, 762, 848 and 539 mm per year, in 2015, 2016, 2017 and 2018, respectively (daily values in Fig. 1). Average annual temperature and cumulative annual precipitation during 1981-2010 (30-year climate normal) were 8.3°C and 746 mm across Denmark (Cappelen, 2019). The soil in the experimental field is defined as a sandy loam, and the topsoil (25 cm) is composed of 90 g clay (<2 μm) kg^{-1} soil, 130 g silt (2–20 μm) kg^{-1} soil and 780 g sand (>20 μm) kg^{-1} soil (Djurhuus and Olesen, 2000). In 2019, the average pH ($CaCl_2$) was 5.6, the C content was 22 g kg^{-1} soil and the total N 1.7 g kg^{-1} soil, with no significant differences between treatments (De Notaris *et al.*, 2021). Detailed information about other chemical and physical soil properties can be found in De Notaris *et al.* (2021).

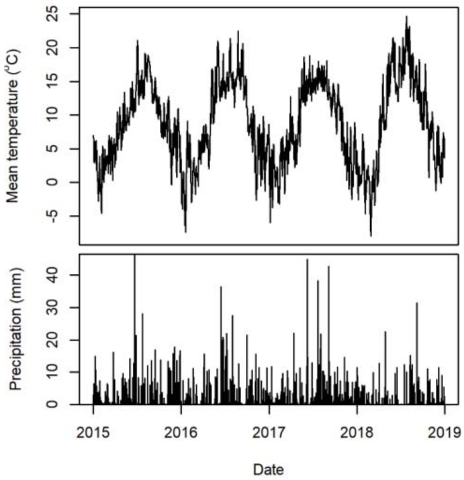

Figure 1: Mean daily temperature and precipitation during 2015-2018 at the experimental site.

2.2 Cropping systems and management

As described in Djurhuus and Olesen (2000), plots in the long-term crop rotation experiment were 12 m wide and 18 m long. The central part (6 × 18 m) of each plot was used as harvest plot, while the two external sides (3 × 18 m) for additional sampling (De Notaris et al., 2021). During the studied period, the 4-year crop sequence consisted of spring barley (*Hordeum vulgare* L.), faba bean (*Vicia faba* L.), spring wheat (*Triticum aestivum* L.) and spring oat (*Avena sativa* L.). The variety of faba bean was Boxer (field bean), a common variety for feed in Denmark. The experiment had a factorial randomized block design with two blocks. All crops in the sequence were represented every year for each treatment (Olesen *et al.*, 2000), which included three factors: cropping system, cover crops and fertilization. Two grain legume-based cropping systems were compared: organic (OGL), with or without legume-based cover crops (CC, NCC) and with and without animal manure (M, NM), and conventional (CGL), with or without non-legume CC (CC, NCC) and with mineral fertilizer (F). Treatment factors were combined with an incomplete design (animal manure was only used for OGL and the combination NM/NCC was not tested), resulting in five treatments, replicated twice for each crop in the rotation: OGL/M/CC, OGL/M/NCC, OGL/NM/CC, CGL/F/CC and CGL/F/NCC.

In all treatments, faba bean did not receive N in fertilizers or manure. In organic treatments, faba bean plots were amended with 300 kg ha^{-1} of Patentkali®, a potash fertilizer approved for organic farming containing 25% potassium (K) as K_2O, 6% magnesium (Mg) as MgO and 17.6% sulfur (S) as SO_3 in water-soluble forms. The same was applied to cereal crops in organic treatments without manure. Cereal crops treated with manure received anaerobically digested animal manure in accordance with common practice for organic farms in Denmark (Plantedirektoratet, 2009), with an average yearly rate of 70 kg total N ha^{-1} for the 4-year rotation. In conventional treatments, faba bean received 75 kg K ha^{-1} in PK (0-4-21) fertilizer, while cereal crops were fertilized with NPK (21-3-10) based on Danish national standards at that time, with an average yearly rate of 110 kg N ha^{-1}, 20 kg P ha^{-1} and 60 kg K ha^{-1} (Plantedirektoratet, 2009). All fertilization operations were performed in April each year.

After harvest, straw was removed from the field. In the organic system, cover crops consisted of a mixture of perennial ryegrass (*Lolium perenne* L.), chicory (*Chicorium intybus* L.), white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.), undersown in May after the last weed hoeing. In the conventional system, cover crops consisted of fodder radish (*Raphanus sativus* L.) for cereal crops (sown in August, after harvest), and ryegrass for faba bean (undersown in May). An exception was 2015, when all cover crops were sown in September with winter rye (*Secale cereal*e L.) and winter rape (*Brassica napus* L.). All cover crops were left in the field during winter and were incorporated in the soil by ploughing in the spring, before sowing of the following crop. Weeds were controlled mechanically in the organic system, while pesticides were used in the conventional to control weeds, pests and diseases, with timing of operations and doses being planned beforehand. The only exception was 2015, when spraying with fungicides in conventional plots was not conducted due to misjudgment. As a consequence, conventional faba bean was damaged by leaf fungal diseases, such as chocolate spot disease (*Botrytis fabae* Sard.), which caused severe yield loss in combination with damages due to a wrongly conducted herbicide treatment. Main field operations in faba bean plots are reported in Table 1.

Table 1: Main field operations and biomass sampling in faba bean plots in 2015, 2016, 2017 and 2018. Fertilizer type, seeding rate and amount of irrigation are reported into brackets, as well as active ingredients of herbicides, insecticides and fungicides, with reference to the main target pest.

Year	Date (m/d/y)	Operation
2015	3/25/2015	Ploughing
	4/17/2015	Fertilization (Patentkali in organic and PK 0-4-21 in conventional)
	4/17/2015	Faba bean sowing (300 kg ha^{-1})
	5/22/2015	Cover crop sowing (10 kg ha^{-1})
	5/26/2015	Spraying with herbicides in conventional (1 l ha^{-1} bentazone (480 g l^{-1}), 1 l ha^{-1} pendimethalin (400 g l^{-1}))
	8/17/2015	Total biomass sampling
	9/11/2015	Cover crop sowing in conventional (58 kg ha^{-1})
	9/29/2015	Faba bean harvest
2016	3/18/2016	Ploughing
	4/01/2016	Fertilization (Patentkali in organic and PK 0-4-21 in conventional)
	4/12/2016	Faba bean sowing (290 kg ha^{-1})
	5/15/2016	Spraying with insecticides against weevils in conventional (gamma-cyhalothrin (60 g l^{-1}), 0.2 l ha^{-1})
	5/21/2016	Cover crop sowing (10 kg ha^{-1})
	6/16/2016	Irrigation (15 mm)
	6/28/2016	Spraying with fungicides against leaf fungal diseases in conventional (boscalid (267 g kg^{-1}) and pyraclostrobin (67 g kg^{-1}), 1 kg ha^{-1})
	8/15/2016	Total biomass sampling
	9/13/2016	Faba bean harvest

2017	4/05/2017	Ploughing
	4/11/2017	Fertilization (Patentkali in organic and PK 0-4-21 in conventional)
	4/11/2017	Faba bean sowing (285 kg ha^{-1})
	5/13/2017	Spraying with insecticides against weevils in conventional (tau-fluvalinate (240 g l^{-1}), 0.2 l ha^{-1})
	5/19/2017	Cover crop sowing (10 kg ha^{-1})
	6/19/2017	Spraying with fungicides against leaf fungal diseases in conventional (boscalid (267 g kg^{-1}) and pyraclostrobin (67 g kg^{-1}), 1 kg ha^{-1})
	8/15/2017	Total biomass sampling
	9/25/2017	Faba bean harvest
2018	4/10/2018	Ploughing
	4/11/2018	Faba bean sowing (284 kg ha^{-1})
	4/25/2018	Fertilization (Patentkali in organic and PK 0-4-21 in conventional)
	5/14/2018	Cover crop sowing (10 kg ha^{-1})
	5/16/2018	Spraying with insecticides against weevils in conventional (tau-fluvalinate (240 g l^{-1}), 0.2 l ha^{-1})
	5/24/2018	Irrigation (22 mm)
	6/6/2018	Irrigation (43 mm)
	6/16/2018	Total biomass sampling
	6/25/2018	Spraying with fungicides against leaf fungal diseases in conventional (boscalid (267 g kg^{-1}) and pyraclostrobin (67 g kg^{-1}), 1 kg ha^{-1})
	7/1/2018	Irrigation (40 mm)
	7/18/2018	Irrigation (40 mm)
	8/22/2018	Faba bean harvest

2.3 Crop yield, biomass sampling and analysis, and crop monitoring

For cereal and faba bean crops, grain yields were determined by harvesting 24 m^2 net plot areas with a Haldrup combine harvester. A near-infrared spectroscopy analyzer was used to determine grain dry matter and N content (Infratec^{TM} 1241 Grain Analyzer, Foss A/S). Every year, approximately one month prior to crop harvest (Table 1), total aboveground biomass was sampled from two 0.5 m^2 subplots in each plot. Plant samples were oven-dried (60ºC for 48 h) to determine dry matter content, then finely milled to determine N content using the Dumas method (Hansen, 1989).

Crop monitoring was conducted by field technicians via visual inspection throughout the growing season, and pictures of each plot were taken prior to crop harvest. However, no actual data on pest and disease pressure was recorded, while weed pressure was assessed every year in early June (GS 59).

2.4 Yield stability

The coefficient of variation (CV) is commonly used to assess yield stability, and it is defined as the standard deviation divided by the mean, expressed as percentage of the mean (Equation 1) (Döring and Reckling, 2018). Recent studies have suggested that crop yields may follow a power-law relationship between the sample variance and the sample mean, leading to lower CV with greater sample mean (Döring *et al.*, 2015). To determine whether the standard CV would be a reliable measure of yield stability, it was checked if grain yield of faba bean and spring cereals followed Taylor's Power Law (TPL), which would indicate that changes in CV are confounded by changes in the mean (Döring and Reckling, 2018). This was done by assessing the correlation between $\log_{10}$(variance) and $\log_{10}$(mean) of grain yield for each crop by treatment, across four years, using the cor.test function in R (Pearson´s correlation). Log_{10}(variance) and $\log_{10}$(mean) were not correlated (Supplementary Fig. S1), thus yield stability was assessed by calculating the CV as:

$$\text{CV (\%)} = 100 \times \frac{standard\ deviation}{mean} \qquad (1)$$

2.5 Biological N_2 fixation in faba bean

Biological N_2 fixation in faba bean was determined using the ^{15}N isotopic dilution method (e.g., McNeill *et al.*, 1994), which is based on the addition of ^{15}N enriched N fertilizer to the soil (isotopic labeling). According to this method, the atom% ^{15}N excess (i.e., the difference between atom% ^{15}N in labeled plant material and ^{15}N natural abundance) in the N_2 fixing plant is compared to a reference non-N_2 fixing plant. The main assumption is that the atom% ^{15}N excess of the reference non-N_2 fixing plant reflects the ^{15}N enrichment of soil N taken up by the legume. This requires that reference and N_2 fixing plants have access to the same soil N pool, i.e., that time course and depth of soil N uptake are the same (Unkovich et al., 2008). A lower atom% ^{15}N excess is expected in the N_2 fixing compared to the reference, because of the dilution effect of the atmospheric N_2 (Danso *et al.*, 1993).

In 2015, 2016 and 2017, two 1 m^2 mini plots for ^{15}N isotopic labeling in each faba bean plot were established. Mini plots were placed each on one side (3 m wide) of the plots, in the central meter, thus being 8 m apart (see section 2.2 for a description of the plot structure). As there was no interference between them, the two mini plots within each plot were considered independent. In this way, biological N_2 fixation in all faba bean treatments was assessed based on four replicates (two plots with two independent mini plots each). Soon after establishment of faba bean, perennial ryegrass was sown between faba bean rows within and outside the mini plots to be used as a reference crop, to ensure that reference and N_2 fixing plant had access to the same soil N pool. In early June, after the last hoeing and the establishment of perennial ryegrass in the mini plots, the soil was enriched with ^{15}N by adding 0.472 g of labeled ammonium sulfate (98 atom%, $(^{15}NH_4)_2SO_4$, corresponding to 1 kg N ha^{-1}). The labeled ammonium sulfate was dissolved in water (10 ml solution), then mixed with 2-3 l of water in an ordinary irrigation can and distributed homogeneously in the mini plots. In early September, a few days before harvest, aboveground biomass of faba bean and ryegrass were sampled from the central part of the mini plots (50×50 cm). To determine ^{15}N natural abundance, samples of faba bean and ryegrass were collected also from areas outside the mini plots. All plant material was dried (60ºC for 48 h) and finely ground with a ball-mill (Mixer Mill MM 400) before being packed in tin capsules (3-4 mg) and sent to UC Davis Stable Isotope Facility for analysis of ^{15}N content by a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd, Chesire, UK).

The percentage of N derived from the atmosphere (%Ndfa) was calculated according to McNeill *et al.* (1994):

$$\%Ndfa = 100 \times \left(1 - \frac{Faba\ atom\%\ 15N\ excess}{Ryegrass\ atom\%\ 15N\ excess}\right) \quad (2)$$

where atom% ^{15}N excess is the difference between ^{15}N atom% in the labeled plant and the plant ^{15}N natural abundance. The N input quantity via biological N_2 fixation (qBNF) in aboveground biomass, defined as the amount of N deriving from fixation, was then calculated as:

$$qBNF = TotN \times \frac{\%Ndfa}{100} \quad (3)$$

where TotN is the total N yield in faba bean aboveground biomass.

2.6 Statistical analysis

Statistical analyses and data exploration, i.e., visual investigation with multi-panel dot plots and boxplots (Zuur *et al.*, 2010), were conducted using R (R Core Team, 2016). Analysis of variance (ANOVA) was used to test the effects of cropping system (organic vs conventional), use of cover crops and animal manure (within the organic system) on crop yield and yield stability (CV) of faba bean and cereals (hypothesis 1), as well as on biological N_2 fixation (%Ndfa) in faba bean (hypothesis 2). Relevant subsets of data were used to minimize possible confounding effects due to the incomplete factorial design. In particular, the effects of cropping system (organic vs conventional) and use of cover crops were tested by comparing OGL/M and CGL/F with and without cover crops. For organic treatments, the effect of manure was tested by comparing treatments with cover crops (i.e., OGL/M/CC vs OGL/NM/CC). Except for CV (which was calculated across years), the interaction effect between each treatment factor and year was tested and, when significant, a post hoc pairwise comparison was performed using the Tukey HSD test. For CV, we tested the effect of crop type (faba bean and cereals) and the interaction with treatment factors (hypothesis 1).

To provide an example of the models used, the effect of cropping system (organic vs conventional) on faba bean grain yield was tested as:

```
dt1 ← dt[dt$Fertilizer!= "NM",]
summary(m1← aov(Yield~CropSyst*Year, data=dt1))
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The assumptions of normality and homoscedasticity were checked using the Shapiro-Wilk test and by visual examination of residuals against fitted values. The correlations between %Ndfa and faba bean aboveground biomass, and between wheat grain N yield and N in faba bean aboveground residues were tested using the cor.test function in R (Pearson's product-moment correlation). For all statistical tests $\alpha=0.05$.

3. Results

3.1 Yield of faba bean and spring cereals

Across all four years (2015-1018), the average faba bean grain yield (expressed as dry matter) varied from 3.7 to 4.8 Mg ha^{-1}, respectively, in OGL/M/CC and CGL/F/NCC (Fig. 2a). Conventional treatments had a significantly greater grain yield compared to organic ($p<0.001$), with a significant interaction effect between cropping system and year ($p<0.001$) (Table 2). In particular, the effect of year was significant in conventional treatments but not in organic ones, with conventional faba bean yield being lowest in 2015 and highest in 2017 (Table 3). Faba bean grain yield was not affected by the long-term use of manure in organic treatments or by use of cover crops (Fig. 2a). Treatment and year did not affect N concentration in faba bean grain, which was on average 4.6%. Therefore, grain N yield followed grain dry matter yield (Fig. 2c). Total aboveground biomass was less affected by treatment than grain yield (Fig. 2b), with only small differences due to cropping system, use of manure and cover crops. Year, however, had a significant effect on total aboveground biomass in all treatments ($p<0.001$), with the greatest average biomass in 2015 (13 Mg ha^{-1}) and the lowest in 2018 (9 Mg ha^{-1}), across all treatments. Total N in aboveground biomass followed the variations in biomass dry matter, with most of the variation being explained by year ($p<0.001$). However, N concentration in total aboveground biomass varied between cropping systems, leading to N in aboveground biomass being significantly greater in conventional compared to organic treatments ($p<0.05$) (Fig. 2d).

As documented in pictures, organic faba bean crops were greatly affected by leaf fungal diseases in late summer, leading to pronounced leaf senescence compared to conventional treatments (Fig. 3 and Supplementary Fig. S2). Average weed pressure in early June was 4% in conventional and 6% in organic treatments without cover crops, while it reached 18% in organic treatments with cover crops (Supplementary Table S2).

Table 2: Analysis of variance (ANOVA) for the effect of cropping system (organic vs conventional), use of cover crops and animal manure (within the organic system) and year on faba bean grain yield and biological N_2 fixation (%Ndfa), as well as the interaction effect between each treatment factor and year. The effect of treatment factors and crop type (faba bean and cereals), plus the interaction with cropping system is reported for the coefficient of variation (CV) of grain yield. For balanced comparisons, within the organic system only treatments with manure were used for testing cropping system and use of cover crops, and only treatments with cover crops for testing the effect of animal manure. Significance is indicated with p values.

	Grain yield	CV	%Ndfa
Cropping system	p<0.001	p<0.001	p<0.001
Cover crop	ns	ns	ns
Manure	ns	ns	ns
Year	p<0.001	-	p<0.001
Cropping system*Year	p<0.001	-	ns
Cover crop*Year	ns	-	ns
Manure*Year	ns	-	ns
Crop type	-	p<0.001	-
Cropping system*Crop type	-	p<0.001	-

ns: not significant; "-": not relevant

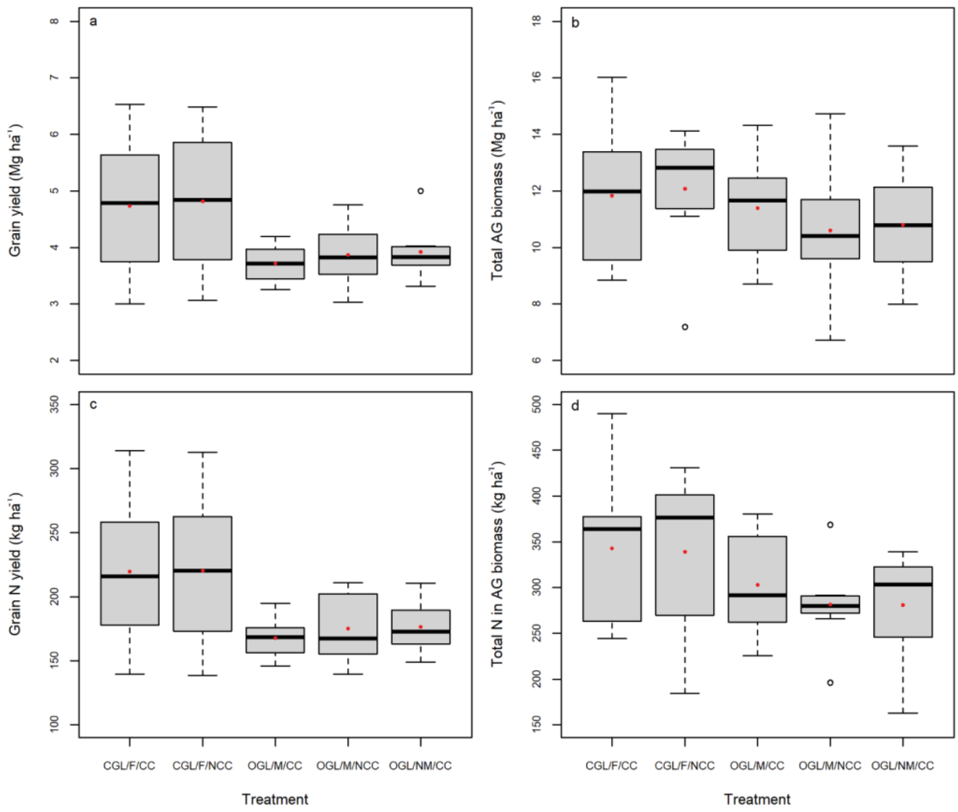

Figure 2: Faba bean grain yield and total aboveground biomass over four years (2015-2018). a) Grain yield at harvest, b) total aboveground biomass one month prior to harvest, c) grain N yield at harvest, d) total N in aboveground biomass one month prior to harvest. Red dots are average values over the four years (n=8). Lines within the boxes represent median values, box boundaries include the 25th and 75th percentiles, and the whiskers extend from the box boundary to the largest and smallest values no further than 1.5 times the inter-quartile range. AG = Aboveground, C = Conventional, O = Organic, F = Fertilizer, M = Manure, NM = No Manure, CC = Cover Crop, NCC = No Cover Crop.

Figure 3: Faba bean in organic (left) and conventional (right) treatment plots during pod filling. Pictures taken by Erling E. Nielsen on August 14, 2017.

Grain yield of all spring cereals was significantly affected by year ($p<0.001$). Barley and wheat had the lowest yield in 2016, whereas oat yield was lowest in 2018 (Table 3). While for faba bean the effect of year was more pronounced in conventional than in organic treatments; for cereals the interaction between these two factors was not significant. Overall, cereals grown in conventional treatments had greater yields compared to organic ($p<0.001$), and there was a significant positive effect of manure application in the organic treatments.

Table 3: Grain yield of spring cereals and faba bean in each treatment and year. Data are mean values (n=2), with standard errors reported into parentheses.

				Grain yield (Mg ha^{-1})			
Cropping system	Fertilizer	Cover crop	Crop	2015	2016	2017	2018

CGL	F	CC	Barley	6.2 (0.01)	3.9 (0.01)	5.9 (0.14)	5.6 (0.47)
			Faba bean	3.1 (0.14)	5.2 (0.06)	6.3 (0.24)	4.3 (0.13)
			Wheat	5.8 (0.11)	5.0 (0.01)	5.5 (0.07)	5.5 (0.19)
			Oat	6.5 (0.07)	6.3 (0.11)	6.9 (0.08)	4.0 (0.19)
		NCC	Barley	6.6 (0.09)	4.6 (0.52)	5.9 (0.21)	6.2 (0.05)
			Faba bean	3.8 (0.43)	5.3 (0.19)	6.4 (0.12)	3.8 (0.77)
			Wheat	6.9 (0.05)	5.2 (0.01)	5.7 (0.10)	6.5 (0.83)
			Oat	6.7 (0.15)	6.2 (0.23)	6.7 (0.00)	4.3 (0.76)
OGL	M	CC	Barley	4.6 (0.56)	2.6 (0.36)	4.0 (0.4)	5.7 (0.53)
			Faba bean	3.9 (0.32)	3.8 (0.42)	3.7 (0.04)	3.5 (0.25)
			Wheat	5.5 (0.22)	4.6 (0.41)	4.5 (0.27)	4.4 (0.34)
			Oat	5.1 (0.13)	5.3 (0.15)	5.5 (0.19)	3.8 (0.45)
		NCC	Barley	4.5 (0.3)	2.0 (0.18)	3.5 (0.25)	4.4 (0.05)
			Faba bean	4.4 (0.34)	3.6 (0.06)	4.2 (0.19)	3.3 (0.26)
			Wheat	4.8 (0.16)	4.0 (0.05)	4.4 (0.23)	4.4 (0.01)
			Oat	5.5 (0.10)	4.6 (0.19)	4.8 (0.12)	3.2 (0.09)
	NM	CC	Barley	3.3 (0.11)	2.4 (0.22)	3.7 (0.23)	4.8 (0.27)
			Faba bean	4.3 (0.66)	3.7 (0.04)	4.0 (0.01)	3.6 (0.28)
			Wheat	4.2 (0.10)	3.3 (0.06)	3.2 (0.13)	3.4 (0.12)
			Oat	5.6 (0.01)	4.4 (0.04)	4.9 (0.25)	2.5 (0.22)

CGL = Conventional with Grain Legume, OGL = Organic with Grain Legume, F = Fertilizer, M = Manure, NM = No Manure, CC = Cover Crop, NCC = No Cover Crop.

3.2 Yield stability of faba bean and spring cereals

Yield stability over four years (2015-2018) varied significantly between crops ($p<0.001$, Table 2) and was highest for wheat and lowest for barley, which had a CV of 10% and 25%, respectively, across all treatments (Table 4). The effect of cover crops on CV was not significant (6 and 15% in CC and NCC, respectively), as well as the effect of animal manure (6 and 12% in M and NM, respectively) in organic treatments. The effect of cropping system (organic vs conventional) varied based on crop (interaction $p<0.001$). No difference between cropping systems was found for wheat and oat, barley had a greater

CV in organic compared to conventional ($p<0.001$), and faba had a greater CV in conventional compared to organic ($p<0.001$) (Table 4). Yield variability in organic faba bean was similar or lower than in organic cereal crops, while CV of conventional faba bean was significantly greater than barley and wheat (Table 3). A CV of 22% for conventional faba bean would be obtained by excluding 2015 from the calculation, which would still be significantly higher than CV of organic faba bean, but not significantly different from the CV of conventional spring cereals.

Table 4: Coefficient of variation (CV) of spring cereals and faba bean grain yield. Mean values were calculated over the years 2015-2018. Mean values averaged over cover crop treatments* (n= 4).

Crop	Cropping system	CV (%)
Barley	OGL	32 a
	CGL	17 cd
Faba bean	OGL	10 d
	CGL	28 ab
Wheat	OGL	9 d
	CGL	10 d
Oat	OGL	19 bcd
	CGL	21 bc

CGL = Conventional with Grain Legume, OGL = Organic with Grain Legume. * Excluding NM treatments in the organic cropping system. Different letters following mean values indicate statistically significant differences ($p<0.05$) between combinations of crop and cropping system.

3.3 Biological N_2 fixation in faba bean

The percentage of N derived from the atmosphere (%Ndfa) at harvest differed significantly between treatments ($p<0.001$) and years ($p<0.001$), with no significant interactions (Table 2). Across all treatments, %Ndfa was lowest in 2016 and highest in 2017. Mean %Ndfa ranged from 73% (O/NM/CC in 2016) to 95% (C/F/NCC in 2017) (Table 5). Overall, %Ndfa was significantly higher in conventional treatments compared to organic with manure ($p<0.001$). In the conventional cropping system, there was a tendency for higher %Ndfa without cover crops, while there was no consistent effect of cover crop and long-term use of animal manure in organic systems (Table 5).

Table 5: Percentage of N derived from the atmosphere (%Ndfa) in faba bean. Data are mean values for each treatment in single years (n=4), with standard errors reported into parentheses, and across all years.

Cropping system	Fertilizer	Cover crop	2015	2016	2017	All years
CGL	F	CC	86 (2)	81 (4)	93 (2)	87 ab
		NCC	91 (1)	90 (4)	95 (1)	92 a
OGL	M	CC	84 (3)	78 (3)	88 (3)	83 b
		NCC	80 (4)	80 (2)	84 (2)	81 b
	NM	CC	82 (2)	73 (5)	88 (3)	81 b

CGL = Conventional with Grain Legume, OGL = Organic with Grain Legume, F = Fertilizer, M = Manure, NM = No Manure, CC = Cover Crop, NCC = No Cover Crop. Different letters following mean indicate statistically significant differences ($p<0.05$) between treatments, across all years.

In the conventional cropping system, %Ndfa was significantly positively correlated to total aboveground biomass ($p<0.05$) with a correlation coefficient of 0.59, while no correlation was found for the organic system (Fig. 4).

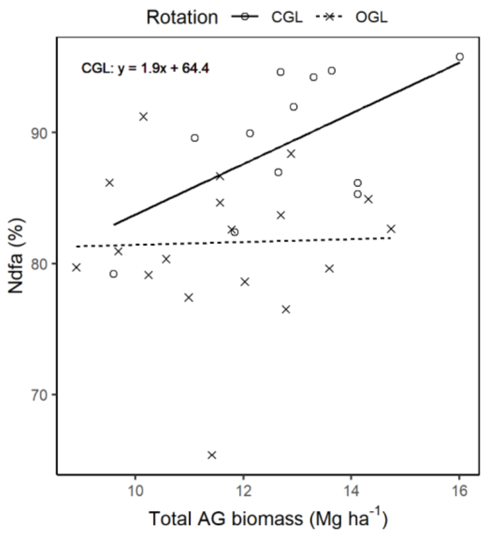

Figure 4: Percentage of N derived from the atmosphere (%Ndfa) in relation to faba bean aboveground biomass. Each data point represents one plot in a single year. AG = Aboveground; CGL = Conventional with Grain Legume, OGL = Organic with Grain Legume.

3.4 Quantity of N input via biological N_2 fixation

The N input quantity via biological N_2 fixation (qBNF) in aboveground biomass differed significantly between treatments ($p<0.01$) and years ($p<0.05$), following changes in %Ndfa (Table 5) and total N yield in aboveground biomass (Fig. 2). Across 2015, 2016 and 2017, qBNF was significantly higher in conventional treatments with 334 kg N ha^{-1} compared to 255 kg N ha^{-1} in organic treatments ($p<0.01$) (Table S1). Across treatments, qBNF was highest in 2017 and lowest in 2016, with 319 and 261 kg N ha^{-1}, respectively. Similar to %Ndfa, use of cover crops and animal manure had no significant effect on qBNF.

4. Discussion

4.1 Productivity and yield stability

In line with our first hypothesis, we found lower faba bean yield in organic than in conventional treatments. Overall, the average faba bean yield across treatments and years was 4.2 Mg ha^{-1}, which is greater than the range of 2.1-3.0 Mg ha^{-1} reported for long-term field experiments in the UK, Sweden, and Germany (Reckling et al., 2018). However, when compared with the same variety (Boxer) grown in field trials in Denmark and Finland, our results are comparable to the average 4.7 Mg ha^{-1} for conventional faba bean treated with herbicides and fungicides (Skovbjerg et al., 2020). This value is very close to the average yield of 4.8 Mg ha^{-1} for conventional treatments in our study. Lower grain yields for organic compared to conventional faba bean can be due to lack of nutrients, such as phosphorous, which can affect the growth of grain legumes directly or indirectly, by impairing growth of rhizobia and the formation and functioning of nodules (Divito & Sadras, 2014). However, all treatments in our long-term field experiment had sufficient levels of phosphorous and other nutrients to sustain faba bean growth (De Notaris et al., 2021). Higher weed pressure could also contribute to lower productivity of organic compared to conventional systems (e.g., Olesen et al., 2007). In our study, organic treatments without cover crops had similar weed pressure as conventional treatments (Supplementary Table S2), and the greater weed pressure associated with the use of cover crops did not correspond to an effect on faba bean grain yield. Therefore, the most likely explanation for the yield gap between organic and conventional management observed in our study was the occurrence of diseases during pod filling (Fig. 3 and Supplementary Fig. S2), which did not compromise the yield of faba bean treated with fungicides in conventional treatments in 2016 to 2018. Organic faba bean plants were affected by leaf fungal diseases, such as chocolate spot, which is caused by the necrotrophic fungus *Botrytis fabae* Sard. and can lead to complete defoliation with mild temperatures and high humidity (Harrison, 1998). These are the typical conditions in late summer in Denmark, when the shoot system of organic faba beans senesced rapidly, with negative consequences for pod filling and grain yield. At similar levels of total biomass accumulation (sampled one month before grain harvest), conventional treatments had greater grain yield compared to organic (Fig. 5c). As chocolate spot was most severe during pod filling, yield loss could be explained by direct damage to pods or by compromised relocation of photoassimilates and nutrients from shoot to seeds (Stoddard et al., 2010). This is further supported by the lower N concentration in organic faba bean aboveground biomass one month before harvest (Fig. 5a), when the organic treatments were already affected by fungal disease. Hence, managing diseases is key to closing the faba bean yield gap between conventional and organic cropping systems.

The gap between N accumulated in biomass one month before harvest and N in grain for the organic system (Fig. 5d) indicates a loss of N and/or that more N is left as residues in the soil in the organic system. Nonetheless, average organic faba bean grain yield was comparable to that of organic spring cereals (Table 3), and it was equally stable across years (Table 4), as also shown by Reckling et al. (2018). Conversely, yield stability of conventional faba bean was lower than conventional spring cereals in our field experiment. Thus, our hypothesis that yield stability of faba bean would be similar to that of spring cereals grown within the same cropping system was only partly verified. By removing disease as a limiting factor, conventional management allowed reaching high yields when environmental conditions were favorable, such as sufficient precipitation throughout the growing season in 2017. However, the high yield potential for conventional faba bean means high exposure to fluctuations from climatic variability, which is expected to increase due to climate change (Webber et al., 2018). Part of the variation in the conventional system could also be explained by the interannual variability in the effectiveness of fungicides, due to difficulties in proper timing of fungicide treatment. In our field experiment, the low yield stability for conventional faba bean was also due to sub-optimal field management in 2015 (section 2.2), which reduced yield in that year. If 2015 was excluded from the calculation, CV of faba bean would be 22 instead of 28% in conventional cropping system, while still 10% in the organic system.

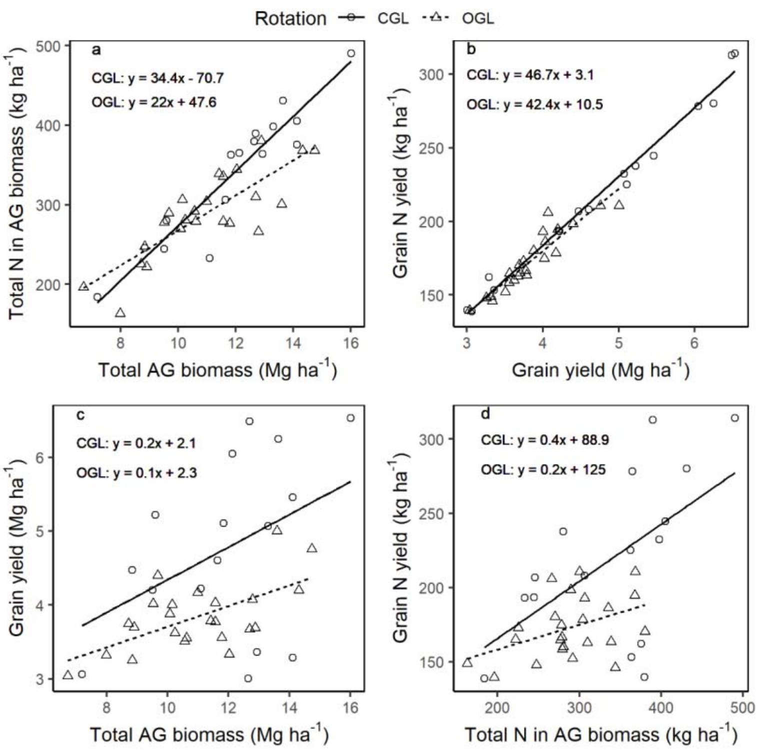

Figure 5: Correlations for faba bean between a) total aboveground biomass and N one month prior to harvest, b) grain yield and N at harvest, c) total aboveground biomass one month prior to harvest and grain yield at harvest, d) total N in aboveground biomass one month prior to harvest and grain N yield

at harvest. Each data point represents one plot in one year. Circles indicate conventional (CGL) plots and triangles organic (OGL) plots. AG: Aboveground.

4.2 Nitrogen yield and biological N_2 fixation

Due to the higher grain N concentration in faba bean (4.6%) compared to cereals (1.8%), faba bean yielded approximately 100 kg N ha^{-1} more than cereals. Since faba bean did not receive any N fertilization, N yield derived from the soil N pool and from BNF. Overall, BNF contributed to more than 70% of N uptake in faba bean, with significant differences between treatments and years. The lowest average %Ndfa was registered in 2016 in the organic treatment with cover crops and without long-term use of animal manure (73%), and the highest was in 2017 in the conventional without cover crops (95%). The overall average %Ndfa in our study (85%) is higher than commonly used standard values, such as 70% (Høgh-Jensen et al., 1998) or 75% (Herridge et al., 2008), but close to values reported in other studies, with %Ndfa higher than 90% for faba bean (e.g., Hauggaard-Nielsen et al., 2009). In a previous cycle of the same long-term crop rotation experiment, %Ndfa in faba bean was estimated to be 61-74% (Pandey et al., 2017) using the N difference method, which relies on quantitative differences in N yield between N_2-fixing and non-fixing plants. That approach is less accurate than the yield independent ^{15}N isotopic dilution method applied in this study, although the latter is also based on assumptions that can be challenging to meet (Unkovich et al., 2008).

Although we expected to find the lowest %Ndfa in organic cropping systems with long-term use of cover crops and animal manure (hypothesis 2), due to the expected positive effect of these practices on soil N availability and the consequent lower N_2 fixation rate (e.g., van Zwieten et al., 2015), there was no consistent effect of cover crops and animal manure on %Ndfa. In conventional treatments, where %Ndfa was greater than for organic faba bean, variations in %Ndfa were significantly and positively correlated with aboveground biomass accumulation (Fig. 4). One reason for this effect could be that, within the same treatment (thus with similar soil N availability), a greater biomass accumulation increased the need for BNF to sustain N requirements, hence the higher %Ndfa (Schulze, 2004). Differences in soil N availability would explain why, at similar levels of total biomass, conventional treatments had higher values of %Ndfa compared to organic. However, the similar soil N status reported for conventional and organic treatments in the same long-term experiment (De Notaris et al., 2021) suggest that the reason for the difference in %Ndfa between conventional and organic treatments could be the overall status of faba bean plants, which were affected by leaf fungal diseases in organic treatments but less in conventional. A lower N_2 fixation rate in organic faba bean could be related to remobilization of N from senescing leaves, which is linked to declined nitrogenase activity (Schulze, 2004).

Overall, calculating aboveground qBNF based on biomass production and %Ndfa for each treatment and year, we obtained values that ranged from 199 to 399 kg N ha^{-1}, which is above average values reported for faba bean (e.g., Watson et al., 2017). This is due to differences in biomass production between varieties (as discussed in section 4.1), as well as variations in %Ndfa. Although it is common to use standard %Ndfa values, such as 70% or 75%, this would have underestimated aboveground qBNF by up to 50 kg N ha^{-1} in organic and 100 kg N ha^{-1} in conventional systems for faba bean grown in our field experiment.

4.3 Perspectives

A correct estimation of how BNF contributes to N balances in cropping systems is essential to fully deploy N services provided by grain legumes (Iannetta et al., 2016). Besides the N exported from the field with grain yield, N in crop residues can be utilized by following crops, reducing the need for N fertilizer. Nitrogen in faba bean aboveground crop residues could be estimated from the difference between total biomass N measured before harvest and N in harvested grain. In our study, wheat N yield was not significantly correlated to N in faba bean aboveground residues in the preceding year, irrespective of cropping system and fertilization (Fig. S3). Unfortunately, a quantification of the residual effect of faba bean in the following crop is not possible in our experiment, as it was not designed with that aim. Nonetheless, it has been shown that inclusion of grain legumes in crop rotations enhances yields of the following crops, with more pronounced benefits in low-input systems (Zhao et al., 2022), such as OGL/NM in our experiment. The lack of a clear correlation between N in faba bean aboveground crop residues and yield of the following crop could be explained by N being lost via leaching (e.g., due to the poor cover crop growth after faba bean in plots with cover crops) or nitrous oxide emissions, or by N build up in soil organic matter (Pugesgaard et al., 2017; De Notaris et al., 2018).

Preissel et al. (2015) estimated that N fertilization to crops following grain legumes can be reduced by 23-31 kg N ha^{-1} due to the contribution of aboveground residues and belowground fractions (roots and deposition in the soil). While aboveground biomass can be easily quantified, belowground fractions are generally estimated by assuming that approximately 30% of the N assimilated by a faba bean plant is found in the roots (Anglade et al., 2015). However, it has been shown that using standard allometric functions is not always reliable, as root:shoot ratios can vary with soil and environmental conditions and management (Hu et al., 2018). For example, it can be expected that different soil conditions and availability of nutrients under organic and conventional management lead to different root growth, with differences between species (Hu et al., 2018; Mortensen et al., 2022). Since under- or overestimating residual N has implications for farmers´ economic return (fertilization and productivity of following crops) as well as environmental and climatic consequences, such as nitrate leaching and N_2O

emissions, using context (e.g., management, soil conditions) specific %Ndfa and belowground values is recommended.

Obtaining high protein yields is a key aim in grain legume production. When it comes to N exported in grain yield, in our study the average protein concentration in faba bean grain was 24.8%, based on the average grain N concentration of 4.6% and the grain legume N-protein conversion factor of 5.4 (Mariotti et al., 2008). However, protein concentrations above 30% have been reported for faba bean grain (Mattila et al., 2018; Meng et al., 2021), with variations due to variety, management, and environmental conditions (Khazaei & Vandenberg, 2020).

Under Northern European conditions, it is common for faba bean to reach maturity late in the growing season, when autumn precipitation can limit field operations and low temperatures can compromise the establishment of cover crops. One solution to this issue, together with the selection of early maturing varieties, would be early harvest of fresh faba bean for human consumption. Besides providing a possible solution to one of the obstacles to growing more grain legumes in Northern European countries, fresh faba bean crop residues could be used for green biorefinery, e.g., to extract protein or refined cellulose. Whereas there is an increasing knowhow regarding extraction and characterization of green protein from forage legumes (e.g., Larsen et al., 2019), the potential of fresh grain legume residues for biorefinery is yet to be assessed. This may be of relevance for cultivation of faba bean in organic farming, where the negative impacts of disease appear primarily at the end of the growing season.

5. Conclusion

Four years of field experiment showed that productivity, yield stability and BNF of faba bean varied with management. Faba bean was more productive in conventional than organic treatments, where the occurrence of pests and diseases limited yield. Conventional faba bean was more exposed to interannual variability, which led to low yield stability. Compared to spring cereals, organic faba bean yield was as or more stable, with comparable average yields.

N derived from atmosphere (%Ndfa) varied from 78% to 93%, and was higher in conventional than in organic systems, with no significant effect of long-term use of animal manure and cover crops. Differences in %Ndfa were driven by crop N requirements related to biomass accumulation, which was greater in conventional treatments, and the negative effect of leaf fungal infection in organic treatments. Using specific %Ndfa and biomass values, the quantity of aboveground N derived from BNF (qBNF) ranged from 199 to 399 kg N ha^{-1}. This would have been greatly underestimated using standard %Ndfa values. Under- or overestimating qBNF has agronomic and environmental consequences, e.g., in the estimation of the residual N effect to following crops and subsequent adjustment of N fertilization. Thus, it is recommended to account for the effect of management on productivity and %Ndfa when assessing faba bean contribution to the efficiency of cropping systems.

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