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Effect of cultivar and inoculant on yields of faba beans (*Vicia faba* minor) and subsequent spring wheat (*Triticum aestivum*) under Scandinavian cropping conditions

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Inoculation of legumes is generally considered to increase yield and to lower the need of nitrogen (N) fertilization, especially in semiarid regions and on sandy soils. It has not been clear whether inoculation with *Rhizobium* sp. in cropping of faba beans (*Vicia faba* minor) under Swedish conditions would improve yield and protein content. In 2015–2016, three faba bean cultivars and two strains of *Rhizobium* were studied in field trials in Central Sweden, including analyses of N fixation capacities using ^{15}N abundance. The study did not show any effects of inoculation of *Rhizobium* on yield or protein content of faba beans or subsequent spring wheat yields. Yields of faba beans varied between cultivars but were not connected to inoculation. ^{15}N abundance was influenced by rhizobium. The study cannot support the opinion that, generally, inoculation is beneficial for improved outcome of faba bean cropping under Scandinavian field conditions. No residual effect of inoculation on subsequent spring wheat yield was found.

KEYWORDS

legume cropping, horse bean, soil improvement, nitrogen, wheat

Introduction

Vicia faba minor (L.), colloquially referred to as faba (or fava) bean, is an ancient legume crop cultivated worldwide for human consumption and livestock feed. Broad bean, horse bean, and field bean are commonly used names for the same species, often distinguished by the seed size (Cubero, 1974; Duc, 1997). The use of faba beans is in Northern Europe is, today, mainly connected to feed, whereas, in Southern Europe, Northern Africa, and the Middle East, it is still a major staple.

Seed yields of faba beans in Scandinavia vary between 3 and 4 metric tons (MT) per hectare (ha) (SJV, 2019; SCB, 2019), and protein content is typically around 30% of dry matter (Klingspor, 2017). The faba bean ability of biological nitrogen fixation (BNF) and its adaptation to various soils and cool climate conditions make it interesting to Scandinavian farmers as a break crop in normally grain-dominated crop rotations.

In Scandinavia, small seed varieties of faba bean (*Vicia faba* minor) have been favored for cultivation, mainly for feed purpose. *Vicia faba* minor has received much attention through development of new low-tannin varieties that are suitable as a protein fodder crop in pig and milk production. The cultivated area of faba beans increased steadily since the 1990s, and, today, the acreage in Sweden varies annually between 15,000 and 30,000 ha (SCB, 2019).

Inoculation of faba bean seeds with N-fixing bacteria has been shown to enhance BNF and to reduce the need for organic or mineral fertilization (Elsheikh and Elzidany, 1997) especially on sandy soils (Abdel-Ghaffar, 1988; Youseif et al., 2017). However, the inoculated strains must be competitive comparing with the native strains (Moawad and Beck, 1991), and the effect of various strains may differ with crop variety (Rodelas et al., 1999; Ntatsi et al., 2018). Furthermore, inoculation of faba beans may increase yields of non–N-fixing crops the following season, as, e.g., seen in crop rotations with wheat in Ethiopia (Habtemichial et al., 2007).

Scandinavian field trials in beans, peas, and lupines in the period 1910 to 1930 showed, in general, a positive reaction on yield and plant development by inoculation—why Swedish agricultural scientist recommended and produced inocula mixtures for farmers (Barthel and Rhodin, 1914; Barthel and Bjälfve, 1930; Bjälfve, 1935). More recent studies on inoculation in various soils and bean types (Rai, 1992; Raposeiras et al., 2006; Uaboi-Egbenni et al., 2010) have shown a general yield increase compared to non-inoculated plots.

It is currently not clear whether inoculation on productive soils in Scandinavia will affect yields and protein content in faba beans. The typically high clay content of Scandinavian soils gives them a better nutrient holding capacity than sandy soils, and inoculation may not be as effective as on lighter soils poorer in nutrients. The fact that cropping of faba beans in Sweden and Scandinavia has been carried out for centuries and thus maintained an active *Rhizobium* flora in the soil may also reduce the need for addition of new strains. Furthermore, it is unclear whether there is an interaction between faba bean cultivars and *Rhizobium* strains, i.e., if some bean varieties will be more affected than others when inoculated with *Rhizobium*.

The aim of this study was i) to investigate the effects of inoculation of faba bean seeds with *Rhizobium* sp. on yield and protein content of faba beans under Scandinavian conditions, ii) to investigate interactions between faba bean cultivars and *Rhizobium* strains, and iii) to investigate residual effects on yield of subsequent spring wheat crop.

In addition, differences in N fixation capacity between cultivars were investigated by analyzing ^{15}N abundance.

Materials and methods

Field experiments

In 2015 and 2016, field experiments were conducted in faba beans (*Vicia faba* minor). Three faba bean cultivars were studied in combination with inoculation with two strains of *Rhizobium* and one control with no inoculation.

The trials were located at Brunnby Farm close to Västerås, Central Sweden (N 59° 36.6001′ E 16° 39.0864′l). The field experiments were seeded and maintained by the regional Rural Economy and Agricultural Society in Västmanland, an organization specialized in field trials and agricultural advisory services (www.hushallningssallskapet.se).

The soil at the experimental site is a heavy clay soil with an organic matter content of 4%–5%. Additional soil properties are shown in Table 1. The soil parameters—pH, nitrate (NO_3)–N, and ammonia (NH_4)–N—were recorded by soil analyses of each treatment after harvest of winter wheat, with soil depth of 0–20 cm. Prior to our experiments, the field had been cropped with winter wheat in 2014 and grass ley in 2013. Weather data are shown in Figures 1, 2.

The trials had a randomized complete block design with four replicates of each treatment (Figure 3). A block thus contained three plots with bean cultivars without inoculation, three plots with bean cultivars inoculated with Swedish standard inoculation, and three plots with new N-fixing strain from Latvia. Each plot had a gross size of 42 m^{-2}. The plots of the field trial were recorded by Global Positioning System (GPS) plotting to use the same plots in the following year for the spring wheat trial.

To investigate the possible residual effects from inoculation of the faba bean seeds, spring wheat (cv. 'Diskett') was seeded on the experimental site in the consecutive year. The wheat crop was harvested, and the yield was registered plot-wise using the same plots previously sown with faba beans.

Three commercial faba bean cultivars available on the Nordic-Baltic market were selected: 'Gloria', a white flowering bean commonly used for feed purpose; 'Julia', a colored flowering bean commonly used for feed purpose; and 'Lielplatones', a white

TABLE 1 Information on soil texture, organic matter content (OM), plant available P (P-Al), plant available K (K-Al), Mg, Ca, Al, and Fe in the topsoil at the experimental sites in 2015 and 2016.

	pH	OM (%)	Clay (%)	Silt (%)	Sand %	P-AL (mg/g)	K-Al (mg/g)	Mg (mg/g)	Ca (mg/g)	Al (mg/g)	Fe (mg/g)
2015	6.0	4.7	55	33	7.3	3.3	17.2	65.5	375	27	51
2016	6.4	4.2	48.0	35.5	12.3	5.2	25.6	51.2	319	35	51

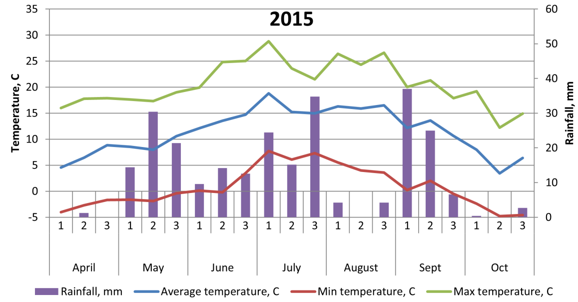

FIGURE 1
Temperature (°C) and precipitation (mm) at Brunnby test site, Sweden, April to October 2015.

flowering bean originating from Latvia. The 'Julia' is considered to have higher tannin content and higher yield than 'Gloria'. 'Lielplatones' has a low Thousand Kernel Weight (TKW) and is thus suitable for seeding with grain seeders. Earlier field cultivar trials in Sweden with 'Lielplatones' have shown a potential for high yield and protein content compared with standard faba bean cultivars used in Sweden.

Inoculation

We used two strains for the inoculations: a commercial standard strain from Inocula Scandinavia AB, Hovmantorp, Sweden; and an experimental strain from Latvia (RV50501) supplied by Dr. Ina Alsina at the Latvian Agricultural University. The Swedish product constituted of a fine-milled peaty powder and the Latvian of a liquid.

The inoculum (5 g of powder or 5 cl of liquid to 500 g of seeds according to instructions by the provider) was applied on the seeds immediately prior to seeding. Beans intended for seeding were placed in a plastic container, and inoculum was added. The beans and inoculum were mixed thoroughly with a plastic stick. We used separate plastic containers and sticks for each mixture of cultivar and inoculum to reduce cross-contamination. Furthermore, to reduce risk of plots being affected by cross-contamination, the seeding machine coulters, hoses, and other parts touched by the inoculated seeds were washed with 95% ethanol between the two

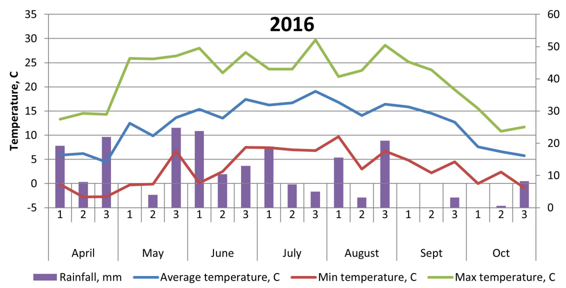

FIGURE 2
Temperature (°C) and precipitation (mm) at Brunnby test site, Sweden, April to October 2016.

different strains. Inoculated plots were also separated by a plot (21 m^{-2}) not used for sampling and a non-seeded strip of 1 m in the beginning of each plot. Plots without inoculation were seeded first.

Field operations

In 2015, beans were seeded on 5 May using a standard plot seeder type "Öyjord". Prior to seeding, the field was harrowed twice using standard methods for the region. The plots were neither fertilized nor treated with herbicides or insecticides. In 2016, beans were seeded in another field nearby on 18 May. As in 2015, the plots were harrowed twice, and neither fertilized nor treated with herbicides or insecticides.

The experiment was harvested at maturity using a field trial combine harvester. For each plot, the yield was registered from a net area of 19.6 m^{-2}. The yield was weighed and dried for later analyses.

In spring 2016, the faba bean trial area of 2015 was seeded with spring wheat cv. 'Diskett' (300 kg of seeds ha^{-1}, row distance of 12.5 cm). On 12 September 2016, the number of wheat heads in the former bean plots was recorded on an area of 0.5 m^{-2} in each plot. Harvest was carried out on 21 September in an area of 19.6 m^{-2} in each former bean plot. In 2017, a similar operation was carried out in the trial area of 2016. A number of heads were recorded on 5 September and yield of spring wheat cv. 'Diskett' was recorded on 28 September 2017. All samples were dried and weighed for further analyses.

^{15}N abundance analysis

To investigate whether N fixation increased by the use of inoculum, a ^{15}N abundance analysis was carried out. This technique quantifies the amount of N derived from N fixation in air versus taken up from the soil. Atmospheric N_2 consists of two stable isotopes, where ^{14}N constitutes ~99.6337% and ^{15}N constitutes ~0.3663%. The ^{15}N abundance analysis is based on the discrimination against ^{15}N uptake over the lighter ^{14}N isotope from atmospheric N_2 in N fixing plants, leading to lower abundance of ^{15}N compared to in non–N_2-fixing plants (He et al., 2009). The ratio between ^{14}N and ^{15}N in the air is constant but varies in other biological systems, for example, in soil where microbial fractionation has an impact (Dijkstra et al., 2006). Delta^{15}N, expressed ($\delta^{15}N$) in parts per thousand ‰, represents the deviation from atmospheric ^{15}N. It is typically a positive value when measured in non–N_2-fixing plants because ^{15}N is more abundant in these plants (Paul et al., 2012).

By comparing $\delta^{15}N$ of inoculated plants and controls, it was possible to determine the effect of inoculation on N fixation.

Samples of the leaves and the stem of faba beans were taken for analyses of ^{15}N abundance in 2015 and 2016, respectively. Ten plants from each plot were collected in July (before pod setting) each year, and the above-ground parts were oven-dried at 106°C for 24 h. The plant material was then ground into <1-mm particle size, and 4 g of biomass of each sample was placed in tin capsules. The analyses were performed by UC Davis Stable Isotope Facility, Davis,

Block IX	X	Lat, Julia	Lat, Gloria	Lat, Lielpl	X	Swe, Julia	Swe, Gloria	Swe, Lielpl	X	0, Gloria	0, Lielpl	0, Julia	X
Block III	X	0, Gloria	0, Lielpl	0, Julia	X	Swe, Lielpl	Swe, Julia	Swe, Gloria	X	Lat, Lielpl	Lat, Gloria	Lat, Julia	X
Block II	X	Swe, Gloria	Swe, Lielpl	Swe, Julia	X	0, Gloria	0, Julia	0, Lielpl	X	Lat, Gloria	Lat, Julia	Lat, Lielpl	X
Block I	X	0, Julia	0, Gloria	0, Lielpl	X	Lat, Julia	Lat, Gloria	Lat, Lielpl	X	Swe, Julia	Swe, Gloria	Swe, Lielpl	X

FIGURE 3
Design of the faba bean field trials in 2015 and 2016 with three inoculation treatments: no inoculation (0), Latvian strain (Lat), and Swedish strain (Swe) in three cultivars of faba beans: Julia, Gloria, and Lielplatones (Lielpl). X = boarder strips sown with untreated faba beans.

California, USA, using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Statistical analysis

The statistical analyses of all trials were carried out by the authors using the Statgraphics Centurion XVI software. Multifactor ANOVA with Tukey's Honest Significant Difference (HSD) was used as standard analysis. All observations and registrations were used in the statistical analyses. Results are presented as mean values ± SE. Statistical differences are presented as p-values using the 95% level as standard.

Results

In the 2015 trial 'Lielplatones' yielded between 5.8 and 6.0 MT ha^{-1} marketable yield, which was significantly higher ($p = 0.0002$) compared to 'Julia' (4.8 to 5.1 MT ha^{-1}) and 'Gloria' (5.4 to 5.6 MT ha^{-1}). There were no statistical differences in yield ($p = 0.8916$) between inoculated and non-treated seeds, regardless of cultivar (Table 2).

TABLE 2 Yields (kg ha^{-1}), protein content (% of DM) and crop stand height (cm) of faba beans in the 2015 and 2016 trials.

Inoculum	Cultivar	Yield		Protein		Crop height	
		2015	2016	2015	2016	2015	2016
0	Julia	5,129 ± 131ab	3,257 ± 168a	28.3 ± 0.30ab	30.3 ± 0.72a	63 ± 1.5ab	87 ± 4.68a
0	Gloria	5,525 ± 131bcd	3,679 ± 168a	29.6 ± 0.30b	28.8 ± 0.72a	59 ± 1.5a	79 ± 4.68a
0	Lielplatones	5,881 ± 131d	3,323 ± 168a	31.0 ± 0.30c	30.0 ± 0.72a	66 ± 1.5b	84 ± 4.68a
Latvian	Julia	5,152 ± 131abc	2,966 ± 168a	29.1 ± 0.30ab	29.5 ± 0.72a	61 ± 1.5ab	84 ± 4.68a
Latvian	Gloria	5,499 ± 131abcd	3,546 ± 168a	29.4 ± 0.30ab	28.9 ± 0.72a	61 ± 1.5ab	71 ± 4.68ab
Latvian	Lielplatones	5,760 ± 131cd	3,704 ± 168a	31.8 ± 0.30c	30.9 ± 0.72a	66 ± 1.5b	89 ± 4.68a
Swedish	Julia	4,885 ± 131a	3,245 ± 168a	28.2 ± 0.30a	29.4 ± 0.72a	61 ± 1.5ab	81 ± 4.68ab
Swedish	Gloria	5,648 ± 131bcd	3,442 ± 168a	28.8 ± 0.30ab	29.7 ± 0.72a	61 ± 1.5ab	82 ± 4.68a
Swedish	Lielplatones	5,985 ± 131d	3,731 ± 168a	31.0 ± 0.30c	29.8 ± 0.72a	66 ± 1.5b	86 ± 4.68a
0		5,512 ± 87a	3,420 ± 76a	29.6 ± 0.19ab	29.7 ± 0.38a	63 ± 0.57	83 ± 2.85a
Latvian		5,470 ± 87a	3,405 ± 76a	30.1 ± 0.19b	29.8 ± 0.38a	62 ± 0.57	81 ± 2.85a
Swedish		5,506 ± 87a	3,472 ± 76a	29.3 ± 0.19a	29.6 ± 0.38a	63 ± 0.57	83 ± 2.85a
	Julia	5,056 ± 87a	3,156 ± 76a	28.5 ± 0.19a	29.7 ± 0.38a	62 ± 0.57	84 ± 2.85a
	Gloria	5,557 ± 87a	3,556 ± 76b	29.2 ± 0.19b	29.2 ± 0.38a	60 ± 0.57	78 ± 2.85a
	Lielplatones	5,875 ± 87b	3,586 ± 76b	31.2 ± 0.19c	30.2 ± 0.38a	66 ± 0.57	86 ± 2.85a
$P_{inoculation}$[a]		ns	ns	0.033	ns	ns	ns
	$P_{cultivar}$[a]	0.001	0.028	0.001	ns	0.001	ns
$P_{inoculation}$ * $P_{cultivar}$[a]		ns	ns	ns	ns	ns	ns

[a]P-values show probability values for the factors inoculum and cultivar and the interaction between these two factors.
Figures with the same letter(s) indicate that there is no statistical difference at the 95% level using Tukey's HSD test. Mean values ± SE.
* = multiplied with, mathematical term.
ns, non significant.

Protein content varied between cultivars: 28.2%–29.1% of DM for 'Julia'; 28.8%–29.6% for 'Gloria', and 31.0%–31.8% for 'Lielplatones', the latter having a significantly higher protein content than the other cultivars (p = 0.0014), regardless of inoculation strains including non-treated (Table 2).

There were generally small differences in crop stand heights (heights varied between 59 and 66 cm). The only significant variation (p = 0.0077) was recorded between 'Lielplatones' regardless of treatment and untreated 'Gloria' (Table 2).

The overall observation of marketable yields was like those of plant height and protein content: small non-statistical differences between 'Julia' and 'Gloria' with 'Lielplatones' generally significantly higher in yield than 'Julia' (Table 2). Furthermore, the analyses did not show any statistical interactions between cultivar and inoculum (treated or non-treated).

Compared to the 2015 trial, the yields were overall lower in 2016. 'Lielplatones' yielded 3.3 to 3.7 MT ha^{-1}, 'Julia' (2.9 to 3.2 MT ha^{-1}), and 'Gloria' (3.4 to 3.6 MT ha^{-1}). There were no statistical differences in yield (p = 0.051) between inoculated and non-treated seeds, regardless of cultivar (Table 3).

Protein content varied slightly between cultivars: on average, 29.7% of DM for 'Julia', 29.2% for 'Gloria', and 30.3% for 'Lielplatones'. There were no statistical differences neither between cultivars (p = 0.17) nor treatments (p = 0.6182).

There were differences in crop stand heights between treatments or cultivars. Plant heights varied between 71 and 87 cm, but none of these variations were statistically separated on the 95% level (p = 0.3037).

^{15}N abundance analysis

The $\delta^{15}N$ values of analyzed faba bean samples ranged from 1.00 to 1.75 in the 2015 trials and from −0.52 to −0.16 in 2016. The $\delta^{15}N$ in controls (non-legume plants) were 6.18 and 6.83, respectively (Table 3). These results clearly show that the bean plants have fixed N from air. The effect is more pronounced in 2016. However, as there are few statistical differences between inoculated and non-inoculated plots, the results do not show that inoculation, in general, improved the uptake of N from the air.

In 2015, there was no statistical difference between treatments (p = 0.182), but a highly significant difference between blocks (p = 0.01). In 2016, the $\delta^{15}N$ values varied significantly between treatments (p = 0.0002). In 2016, the varieties 'Gloria' and

TABLE 3 Nitrogen fixation ($\delta^{15}N$) in different cultivars in presence or no presence of inoculum from either Latvia or Sweden in the field trials of 2015 and 2016.

Inoculum	Cultivar	$\delta^{15}N$	
		2015	2016
0	Control	6.180	6.830
0	Julia	1.270 ± 0.17ab	−0.365 ± 0.049bc
0	Gloria	1.750 ± 0.17b	−0.217 ± 0.049d
0	Lielplatones	1.274 ± 0.17ab	−0.285 ± 0.049bcd
Latvian	Julia	1.502 ± 0.17ab	−0.520 ± 0.049a
Latvian	Gloria	1.354 ± 0.17ab	−0.162 ± 0.049d
Latvian	Lielplatones	1.224 ± 0.17a	−0.192 ± 0.049d
Swedish	Julia	1.002 ± 0.17a	−0.4225 ± 0.049 ab
Swedish	Gloria	1.194 ± 0.17a	−0.157 ± 0.049d
Swedish	Lielplatones	1.138 ± 0.17a	−0.275 ± 0.049cd
0		1.431 ± 0.11a	−0,289 ± 0.02a
Latvian		1.360 ± 0.11a	−0,341 ± 0.02a
Swedish		1.111 ± 0.11a	−0,284 ± 0.02a
	Julia	1.258 ± 0.11a	−0,436 ± 0.02a
	Gloria	1.433 ± 0.11a	−0,179 ± 0.02b
	Lielplatones	1.212 ± 0.11a	−0,251 ± 0.02b
$P_{inoculation}$ [a]		ns	ns
	$P_{cultivar}$ [a]	ns	0.001
$P_{inoculation} * P_{cultivar}$ [a]		ns	ns

[a]P-values show probability values for the factors inoculum and cultivar and the interaction between these two factors.
Figures with the same letter(s) indicate that there is no statistical difference at the 95% level using Tukey's HSD test. Control values originates from non–N-fixing plants.
* = multiplied with, mathematical term.
ns, non significant.

'Lielplatones' fixed N to a higher degree compared to Julia' (p = 0.001) independent of inoculation strategy (inoculation *vs.* no inoculation and Swedish *vs.* Latvian).

Statistical analyses over inoculation treatments showed no significant differences between the two inoculum products (p = 0.75) and no statistical difference between the products and non-inoculated plots (p = 0.98).

Effects of preceding bean cultivar and inoculation on spring wheat yields

To evaluate the effects of the faba beans and the two inoculums on the subsequent crop, we analyzed yields, number of heads, and protein content of spring wheat cropped in the previous plots of the bean trials. In the 2016 trial, wheat yields varied between 6.0 and 6.4 MT ha^{-1} and the number of heads varied between 527 and 586 (Table 4). There were no statistical differences in number of head (p = 0.2321), but marketable yield varied between cultivars (p = 0.036) and between inocula (p = 0.001). We registered a significant interaction between cultivar and inocula (p = 0.003).

The results of the 2017 trial were similar to those of 2016 (Table 4). There were no significant differences in wheat yield, number of head, or protein content. The number of heads m^{-2} varied between 498 and 558 (p = 0.3745), yields varied between 5.8 MT and 6.2 MT ha^{-1} (p = 0.3933), and protein content varied between 11.2% and 11.6% of dry matter (DM) (p = 0.4343). Top soil concentrations of mineral N after harvest of winter wheat are presented in Figures 4, 5.

Discussion

Inoculation of legumes is generally carried out to improve the N_2 fixation of the legume plants and thereby reduce the need for application of mineral or organic fertilizers (Amanuel et al., 2000; Dubova et al., 2015). Growing legumes can in some regions enhance yields of a following crop (Cuthforth et al., 2007; Habtemichial et al., 2007). A range of studies (e.g., Abdel-Ghaffar, 1988; Raposeiras et al., 2006; Uaboi-Egbenni et al., 2010; Youseif et al., 2017; Liu et al., 2019; and Guinet et al., 2020) have shown that inoculation of legumes such as faba beans, common beans, chickpeas, lentils, and field peas will result in, e.g., increased yields of these crops, increased N-content in soil, and increased yields of the succeeding crop. However, its known that nodulation and biomass production depend on the compatibility between faba

TABLE 4 Yields (kg ha^{-1}) and number of heads (m^{-2}) of spring wheat in 2016 and 2017 using the same plots as used for growing faba beans in 2015 and 2016, respectively.

Inoculum	Faba bean cultivar	Yield		No. of wheat heads	
		2016	2017	2016	2017
0	Julia	6,645 ± 82d	5,803 ± 90a	528 ± 15.6a	506 ± 13.1a
0	Gloria	6,120 ± 82abc	5,842 ± 90ab	548 ± 15.6a	544 ± 13.1a
0	Lielplatones	6,217 ± 82bc	6,007 ± 90ab	552 ± 15.6a	498 ± 13.1a
Latvian	Julia	5,900 ± 82ab	6,020 ± 90ab	536 ± 15.6a	512 ± 13.1a
Latvian	Gloria	6,110 ± 82abc	6,090 ± 90b	542 ± 15.6a	538 ± 13.1a
Latvian	Lielplatones	5,758 ± 82a	5,988 ± 90ab	532 ± 15.6a	520 ± 13.1a
Swedish	Julia	6,222 ± 82c	5,969 ± 90ab	554 ± 15.6a	536 ± 13.1a
Swedish	Gloria	6,415 ± 82cd	5,937 ± 90ab	586 ± 15.6a	558 ± 13.1a
Swedish	Lielplatones	6,324 ± 82cd	5,918 ± 90ab	528 ± 15.6a	530 ± 13.1a
0		6,327 ± 40b	5,884 ± 32.4a	542 ± 8.6a	516 ± 7.7a
Latvian		5,923 ± 40a	6,033 ± 32.4a	536 ± 8.6a	523 ± 7.7a
Swedish		6,320 ± 40b	5,949 ± 32.4a	556 ± 8.6a	541 ± 7.7a
	Julia	6,256 ± 40a	5,931 ± 32.4a	539 ± 8.6a	518 ± 7.7a
	Gloria	6,215 ± 40ab	5,964 ± 32.4a	558 ± 8.6a	546 ± 7.7a
	Lielplatones	6,099 ± 40b	5,971 ± 32.4a	537 ± 8.6a	516 ± 7.7a
$P_{inoculation}$[a]		0.001	0.002	ns	ns
	$P_{cultivar}$[a]	0.036	ns	ns	ns
$P_{inoculation}$ * $P_{cultivar}$[a]		0.003	ns	ns	ns

[a]P-values show probability values for the factors inoculum and cultivar and the interaction between these two factors.
Figures with the same letters are not statistically separated at the 95% level using Tukey's HSD test.
* = multiplied with, mathematical term.
ns, non significant.

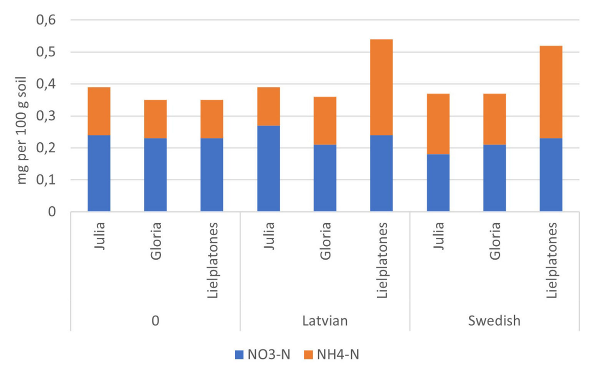

FIGURE 4
Soil mineral–N (g 100 g jord^{-1}) in the top soil (depth of 0–20 cm) after harvest of winter wheat (2016) following on faba bean (2015).

bean genotype and rhizobium strain and its interaction with soil bio-physical conditions (Allito et al., 2021). Many studies, including those mentioned above, have focused on *Rhizobia* to enhance yields and to reduce the need of mineral fertilization in arid or semiarid regions (Allito et al., 2020), saline-sodic soils (Rai, 1992), acid soils (van Zwieten et al., 2015), or on regions previously not commonly cropped with faba beans. There are few recent studies on the effect of inoculation of common beans or faba beans under North European conditions. Early works by Barthel and Bjälfve (1930) and Bjälfve (1935) are still used as a basis for inoculation of legumes in Scandinavia. The results of our trials did, however, not support these early North European studies where inoculation of faba bean seeds improved the bean yield and accumulated N into the system as well as increase the yield of a subsequent crop. Furthermore, our ^{15}N results show that native rhizobia present in the soil of non-inoculated plots were as effective as the inoculated strains in sustaining BNF, a finding also discussed by Irisarri et al. (2019).

The ^{15}N abundance analyses of our trials showed that the ability to fix atmospheric N is not dependent on cultivar or presence of inoculum. Legumes, including *Vicia faba*, are known N-fixing crops, whereas the chosen controls in the trials are not able to fix atmospheric N. Thus, δ^{15}N was significantly higher in the controls as expected. These results indicate that use of inoculum under Swedish cropping conditions does not increase N fixation and implies no effect on yields or subsequent crops.

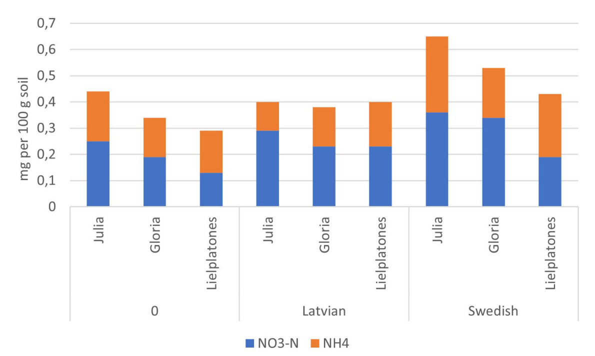

FIGURE 5
Soil mineral–N (g 100 g jord^{-1}) in the top soil (depth of 0–20 cm) after harvest of winter wheat (2017) following on faba bean (2016).

In contrast to our results, Denton et al. (2017) noticed an increase in faba bean yield from 0.48 to 1.94 MT ha^{-1} using various inoculation products. However, this increase was noticed in eastern Australia where seeding normally takes place in dry soils prior to seasonal rains and legume cropping is an exception in the crop rotation. Sweden has a long tradition of cropping peas and faba beans, which likely has developed and maintained the BNF in the soils to a higher extent than in many other areas such as in Australia and parts of the Middle East. In line with our results, Zhang et al. (2010) reported a lack of yield increase from *Rhizobium* inoculation of bean, and they attributed this to a relatively high level of native rhizobia in the soil that annulled the effect of rhizobium inoculation.

Moreover, the N content in Swedish clay soils (a dominating soil type in Sweden and the soil type in the field trials) is generally high compared to in sandy soils in the Middle East or Northern Africa—a consequence of both soil type and climatic conditions. With more N in the soil, a somewhat lower effect of inoculation on the N-fixation and on yield could be expected, as the plant need for fixed N will be less pronounced. Moreover, Liu et al. (2019) reported that even a higher amount of BNF does not always correlate to a higher yield of seeds.

The cultivar 'Julia' generally yielded less than both 'Gloria' and 'Lielplatones', which was somewhat unexpected. Official statistics from cultivar testing in Sweden 2016 (Klingspor, 2017) shows that the average yield (MT ha^{-1}) is 4.8 for 'Julia' and 4.6 for 'Gloria'. White flowering cultivars are considered to yield less, in general, compared to multi-colored cultivars. The overall lower yields in our trials in 2016 are most likely connected to differences in weather conditions upon flowering (Figures 1, 2) with 26.2-mm precipitation in July 2016 compared to 39.6 mm in July 2015. Although faba beans have a deep tap root, plants are favored by cooler and wetter seasons due to a high soil water requirement. A drier season will also affect nutrient uptake negatively. As the trials were not fertilized, only nutrients already present in the soil or fixed from the air were available for the crop stand. Studies by Ntatsi et al. (2018) carried out in Greece on faba beans for fresh seed production investigated a wide range of factors influencing crop yield. Basically, their findings showed that protein content, pod length, and total yield were influenced by bean variety, whereas cropping system (conventional/organic farming) had a minor effect. Finnish studies (Lizarazo et al., 2015) found significant interactions between year and faba bean variety on protein content of the beans, probably connected with genotypic differences in N metabolism. In our study, there were no interactions between year and faba bean varieties on yields or protein content. This might be an effect of our choice of cultivars, soil conditions at the test site, and weather conditions in 2015–2016.

The two tested inocula did not affect yields, number of heads, nor protein content in the kernels of the subsequent spring wheat crop in 2017, but we did register the effects of faba bean cultivar as well as inocula on wheat yield in 2016. Although statistically significant, the result is inconclusive; the Latvian strain seemed to lower overall yields of wheat, whereas the Swedish inocula did not affect yields compared to untreated plots. Although the BNF of the faba bean crops most likely supplied the wheat with N, there seemed to be no increase in N supply by inoculation. These results support the lack of inoculation effect on BNF measured in the faba beans by the use of ^{15}N. Soil type and season had probably a higher effect on wheat yield than choice of bean cultivar or inoculum product used in a previous crop and year.

Conclusions

Sweden has long history of faba bean cropping that most likely has resulted in natural and native *Rhizobium* strains in the soil. For Swedish conditions, there are few, if any, reasons to inoculate with rhizobia at cropping of faba beans in conventional agriculture. However, in the case that faba beans are seeded on sandy soils with low levels of plant available N and previously not known to be cropped with any legumes, an addition of commercially available *Rhizobium* can be beneficial.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

FF: Conceptualization, Methodology, Validation, Writing- Original draft preparation, Writing - Review and Editing. JÖ: Validation, Investigation, Writing- Original draft preparation, Writing - Review and Editing. ÅM: Validation, Investigation, Writing- Original draft preparation, Writing - Review and Editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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