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Soil compaction and insect pollination modify impacts of crop rotation on nitrogen fixation and yield

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Abstract

Pollination and biological nitrogen fixation are key ecosystem services, but their contribution to agricultural production might be influenced by simplified crop rotation and soil compaction, two factors known to limit yield. In a greenhouse experiment, we investigated the combined effect of crop rotation, soil compaction, and insect pollination on yield formation and on the contribution of biological fixation to nitrogen acquisition of faba bean. Seed yield was reduced under high soil compaction, and under ley rotation management and it was enhanced by insect pollination. For plants grown in soil from the ley rotation, insect pollination increased individual seed weight by 50% suggesting a contribution to seed quality by pollination for crop grown in soils where nutrients are limiting yield. Crop monoculture and high soil compaction interactively reduced the contribution of nitrogen fixation by 30%, suggesting that soil compaction exacerbates the negative effect of monoculture on nitrogen fixation.

Overall the results revealed that interactive effects of management factors do affect nutrient acquisition. We provide evidence that reduced soil quality affect the capacity of legumes to deliver key ecosystem services to the agroecosystem.

Zusammenfassung

Bestäubung und biologische Stickstofffixierung sind wichtige Ökosystemleistungen, aber ihr Beitrag zur landwirtschaftlichen Produktion könnte durch vereinfachte Fruchtfolge und Bodenverdichtung beeinflusst werden, zwei Faktoren, die den Ertrag begrenzen. In einem Gewächshaus untersuchten wir experimentell den gemeinsamen Effekt von Fruchtfolge, Bodenverdichtung und Bestäubung durch Insekten auf den Ertrag und auf den Beitrag der biologischen Fixierung zum Stickstofferwerb der Ackerbohne. Der Samenertrag war bei hoher Bodenverdichtung und bei Fruchtfolge mit Grünbrache reduziert, während er durch Insektenbestäubung erhöht wurde. Bei Pflanzen, die in Erde aus der Brachenfruchtfolge wuchsen, erhöhte Insektenbestäubung das individuelle Samengewicht um 50%, was nahelegt, dass Bestäubung bei Böden mit limitierendem Nährstoffangebot einen Beitrag zur Samenqualität leistet. Monokultur und hohe Bodenverdichtung reduzierten gemeinsam den Beitrag der Stickstofffixierung um 30%. Insgesamt zeigten die Ergebnisse, dass interagierende Effekte von Managementfaktoren den Nährstofferwerb beeinflussen. Wir erbringen den Nachweis, dass reduzierte Bodenqualität die Fähigkeit von Leguminosen beeinflusst, entscheidende Ökosystemleistungen für Agrarökosysteme zu erbringen.

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Introduction

Maintaining high and sustainable crop yields is a major challenge for agricultural production. Widely implemented practices such as the simplification of crop rotation (Bennett, Bending, Chandler, Hilton, & Mills 2012) and the intensive use of heavy farm machinery leading to soil compaction (Hamza & Anderson 2005) can reduce nutrient availability (Ball, Bingham, Rees, Watson, & Litterick 2005) and explain declines or stagnation of yield in key agricultural regions (Lin & Huybers 2012; Ray, Ramankutty, Mueller, West, & Foley 2012). However, ecosystem services (ES) provided by biodiversity above and below ground offer opportunities to maintain yield growth and stability (Bommarco, Kleijn, & Potts 2013). For instance, direct contributions of biological nitrogen fixation (BNF) and insect pollination to crop yield have become widely recognized (Klein et al. 2007; Peoples et al. 2009; Garibaldi et al. 2013). Nonetheless, it is not clear whether interactive effects between management factors might affect nutrient acquisition and the contribution of ES to crop yield.

Estimations of the contribution of an ES to crop yield most often assume all other production factors as optimal (Bos et al. 2007); a possibly unrealistic assumption as it does not account for that multiple ES, management, and resources might interactively influence yield formation (Boreux, Kushalappa, Vaast, & Ghazoul 2013; Lundin, Smith, Rundlöf, & Bommarco 2013; Klein, Hendrix, Clough, Scofield, & Kremen 2015). For instance, in oilseed rape, the pollinators' contribution to yield was greater in the absence of nitrogen fertilization, compensating for yield losses associated with the lower nutrient availability (Marini et al. 2015). There is a need to determine if such compensation can occur when nutrient acquisition is limited by management factors.

Crop rotations that include perennial legumes can enhance levels of soil organic matter (Cuvardic, Tveitnes, Krogstad, & Lombnæs 2004; Grover, Karsten, & Roth 2009), provide better soil structure (Ball et al. 2005) and higher N-mineralization (van Eekeren et al. 2008) than monoculture and short rotations. Soil compaction, on the other hand, has been shown to reduce root growth, water and nutrient uptake and thereby decreasing yields (Lipiec & Hatano 2003). We need to reconsider the role of ES to maintain yields, and prevent negative side-effects associated with intensive agricultural management.

Services provided below ground influence plants directly, for example, by affecting nutrient availability (Scheu 2001; Wardle et al. 2004). A widely recognized contribution of belowground biodiversity to plant nutrients is the mutualistic association between roots and nitrogen-fixing bacteria (Peoples et al. 2009). Nitrogen acquisition by legumes is partitioned between soil mineral N absorbed by the roots and N derived from BNF. Since BNF allows legumes to have access to different N sources and since management factors affect the availability of this nutrient, there is a need to investigate how N acquisition is partitioned by plants grown under contrasting soil management factors. Besides, the nodulation trait in legumes has been shown to increase pollen length and germination rate when compared to a non-nodulating mutant (Gwata, Wofford, Pfahler, & Boote 2003); a finding suggesting that BNF modulates the outcome of pollination on yield (Barber & Gorden 2014). However, information is missing on how insect pollination influences BNF at the plant level and whether this is modified by cropping practices. So far, no study has investigated the combined effect of pollination and yield limiting cropping practices on the proportion of N derived from BNF by crops. Large knowledge gaps remain of how crop management and ES interactively determine yields.

To unravel the potential interactive effects of pollination, management type, and soil compaction on crop yield and BNF, we experimentally manipulated these factors in a fully-factorial greenhouse experiment. We measured yield components, growth partitioning, and the proportion of N derived from BNF in faba bean (*Vicia faba* var. *minor* L.), a widely cultivated legume used as a protein source and green manure in mixed cropping systems (Jensen, Peoples, & Hauggaard-Nielsen 2010) and partly dependent on pollination for seed set (Cunningham & Le Feuvre 2013). We collected soil from a long-term experiment that had been managed since 1965 either as a barley monoculture, or as a crop rotation including five years of perennial leys (Puentes, Bazely, & Huss-Danell 2007). We subjected these two soils to a low or high compaction treatment. We subsequently bagged the inflorescences on half of the plants and introduced bumblebees to the greenhouse. We hypothesized that (i) in the presence of pollinators, the negative effects of soil compaction on yield will be less pronounced in soil from the ley rotation; (ii) a higher investment from the plant in seed yield would lead to a lower investment on vegetative parts; (iii) N derived from BNF will be more limited in soils with a combination of high soil compaction and monoculture origin; (iv) since insect pollination increases plant demand for N, pollination will increase the amount of N derived from BNF. Understanding how management factors affect the delivery of ES in agricultural systems will help make yield predictions that take into account current management practices in highly productive regions.

Materials and methods

Study system

To test the impact of management type, soil compaction and pollination on yield and nitrogen fixation of faba bean (*Vicia faba* var. *minor* L.) we set up a greenhouse experiment. The soil used in the experiment was a silty loam collected from a long-term agricultural field experiment located in Röbäcksdalen, Sweden (63°45′ N, 20°17′ E), started in 1965 and managed by the Swedish University of Agricultural Sciences (SLU). We selected two contrasting treatments: a monoculture of barley (*Hordeum vulgare* L.) and a

Table 1. Major soil characteristics measured prior to the start of the greenhouse experiment for soil originating from the barley monoculture and from the ley rotation.

Management type	SOC (%)	Total N (%)	P-AL (mg/100 g)	K-AL (mg/100 g)
Barley monoculture	2.67	0.174	8.04	16.38
Ley rotation	3.63	0.247	6.98	8.11

ley-dominated six-year rotation with undersown barley followed by five years of ley. In each treatment, we collected 200 L of soil from the upper 30 cm of the soil. The species mixture and proportions seeded in the ley consisted of a legume: red clover (*Trifolium pratense* L.) 32%, and two grasses: timothy (*Phleum pratense* L.) 42% and meadow fescue (*Festuca pratensis* Huds.) 26%. Soil characteristics were assessed (Table 1). Soil organic carbon (SOC) and total (N) were determined with an elemental analyzer (LECO CHN 1000). Plant available phosphorus (P) and potassium (K), two macro-nutrients important for faba bean development, were extracted with ammonium lactate solution (Egnér, Riehm, & Domingo 1960).

Experimental setup

The experimental unit consisted of a single faba bean plant sown in PCV tubes (400 mm long, 110 mm inner diameter) and grown in a greenhouse. On April 22nd, 2014, seeds of faba bean of the variety Fuego (Lantmännen, Sweden) were inoculated with commercial inoculum (*Rhizobium leguminosarum*) to insure uniform nodulation and two seeds per tube were planted at a depth of 2 cm. On May 2nd, when plants had 2 leaves unfolded (BBCH developmental stage 12, Lancashire et al. 1991) the smaller of the two seedlings was removed. All plants received the same volume of water throughout the experiment.

Prior to the start of the experiment, the soils from the 2 management types were separately homogenized with a concrete mixer (Flex-Elektrowerkzeuge GmbH, Germany) and passed through a 6 mm sieve to remove coarse fragments and plant residues. The soils were left to dry at 24 °C for 48 h. The resulting soil water content was 0.25 and 0.31 g g^{-1} in soil from the monoculture and rotation, respectively. The PVC tubes were prepared according to Löfkvist, Rydberg, and Svantesson (2000) by coating the inside of each tube with a mixture of glue and sand to prevent roots escaping compaction to the sides. Three kilograms of moist soil were added to each tube and compacted in successive layers of 3 cm thickness, using a pneumatic cylinder (Löfkvist, Whalley, & Clark 2005). In a pilot experiment we measured growth parameters: root length, rooting depth, nodule formation and stem length of faba bean. The low compaction level 50 kPa was chosen because it presented the highest growth parameters. For the high compaction level, 400 kPa was chosen because it presented a reduction in growth parameter similar to those observed for faba beans in the compacted

treatment of a field experiment (Brereton, McGowan, & Dawkins 1986).

We established 80 experimental units (2 management types × 2 compaction levels × 2 pollination levels × 10 blocks, Fig. 1). The treatments were randomized within the blocks and the blocks were randomly distributed in the greenhouse.

When the beans began to flower on the June 1st, 2014, the non-pollination treatment was initiated by bagging the inflorescences of half of the plants with 2 mm-pore bags to prevent pollinators accessing the flowers (Kearns & Inouye 1993). Each bag was a tube of 15 cm diameter, its length depending on the spread of the inflorescences along the stem. Bags were adjusted every day to account for plant growth. Between June 5th and July 20th a hive of bumblebees (*Bombus terrestris* L.) (MINIPOL beehive, Koppert Biological Systems, NL) was present in the greenhouse. The flight hole of the hive was opened every morning at 10:00 to release the bumblebees and closed at 15:00. To account for possible bag effects on the plants, open bags were placed on the pollinated plants when the pollinators had returned to the hive.

Response measurements

### Seed yield, yield components, and growth partitioning

Plants finished flowering on June 26th and were harvested on July 22nd, at the end of the ripening stage when around 80% of pods were ripe and dark, BBCH stage 88 (Lancashire et al. 1991). The aboveground part of each plant was cut at the base of the stem, and dried at 65 °C for 48 h. Once dried, the pods were removed.

Seed yield of faba bean was assessed as total dry weight of seeds per plant. Yield components assessed were: number of seeds per plant, number of pods per plant, number of seeds per pod, individual seed weight and seed set. The individual seed weight was obtained by dividing the seed weight by the number of seeds per plant. Seed set was calculated as number of seeds per plant/number of pods per plant. The plants were weighed to obtain aboveground biomass.

The PVC tubes, in which the plants were grown, were cut open on the side and 15 g of soil was taken from each tube 4 cm from the root system for analysis of total nitrogen (Tru-Mac Series Macro Determinator, Leco Corporation, USA). The tubes were immersed in cold water in a dark room at 5 °C for 24 h. The roots were washed, dried at 65 °C for 48 h and weighed to obtain belowground biomass. Growth partitioning was estimated by separately quantifying total biomass,
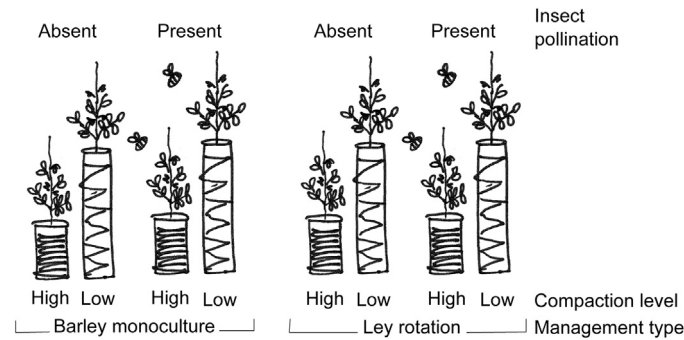
Fig. 1. Schematic representation of the experimental design with soil from two management types (barley monoculture and ley rotation), two compaction levels (low and high), and two pollination levels (insect pollination absent and present).
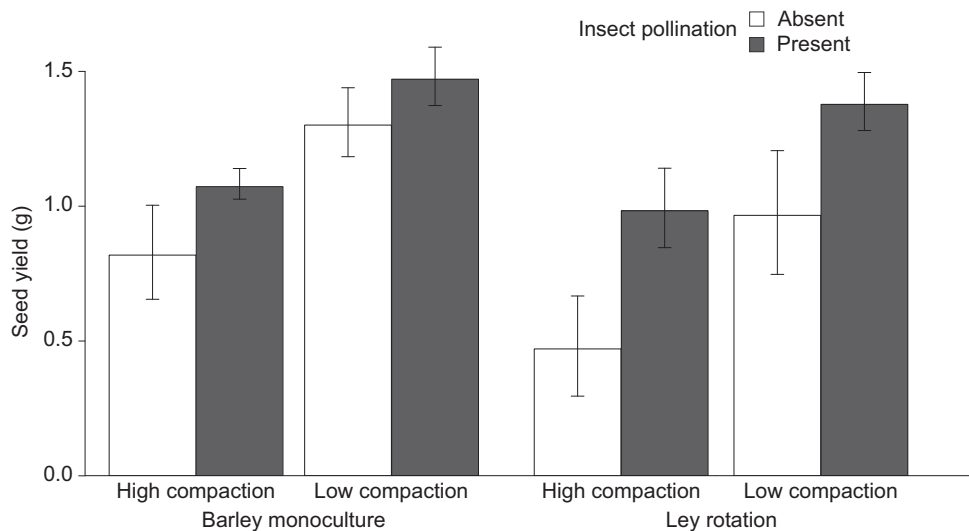
Fig. 2. Effect of management type, soil compaction, and insect pollination treatment on seed yield of faba bean. Means ± standard error are shown ($n = 79$) (see Appendix A: Table 1).
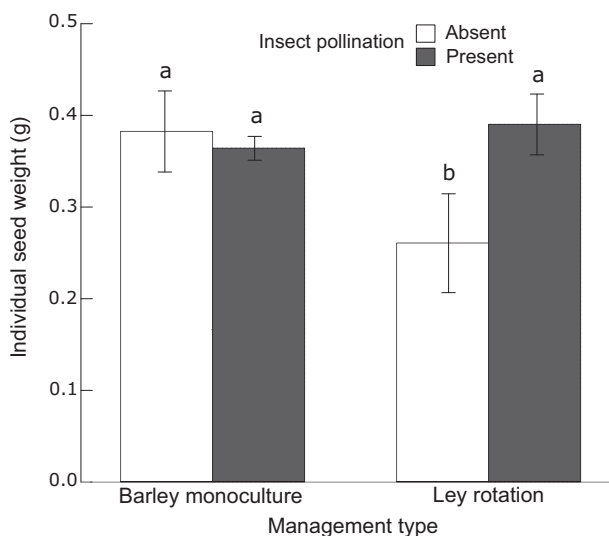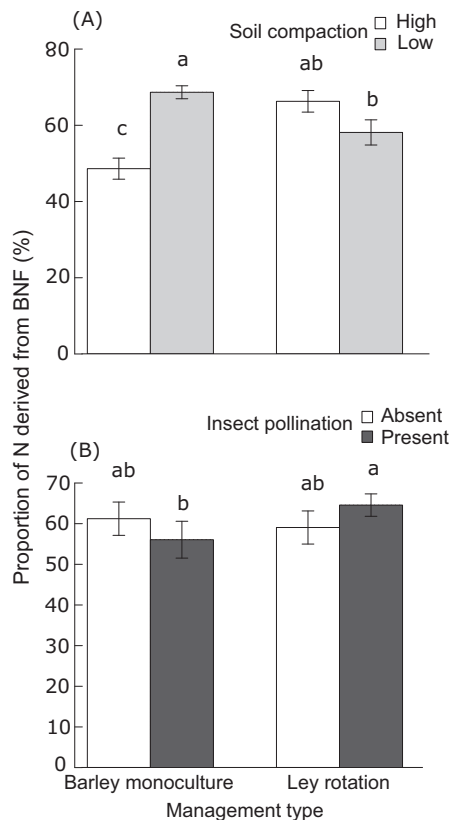

Fig. 3. Effect of management type and insect pollination on individual seed weight. Means ± standard error are shown ($n = 79$) (see Appendix A: Table 1). Bars with identical letters are not significantly different at $P < 0.05$ (Tukey HSD).

aboveground biomass, belowground biomass, root-to-shoot ratio and reproductive-to-vegetative ratio. Reproductive-to-vegetative ratio was calculated as weight of seeds per plant divided by the sum of above- and belowground biomass.

Proportion of N acquisition derived from BNF and plant nitrogen concentration

We estimated the proportion N acquired by faba bean via BNF using the natural abundance method (Shearer & Kohl 1986). At the start of the experiment we prepared 12 extra pots of the compaction and rotation treatments in which barley of the variety Scandium (Lantmännen, Sweden) was sown. The barley pots were randomly distributed within the blocks in the greenhouse and managed in the same way as the faba bean throughout the experiment. Above- and belowground parts of the barley plants were harvested at the same moment as the faba beans. After drying, the above- and below-ground parts of each plant were pooled together and total N, plant N concentration and the natural abundances of ^{14}N and ^{15}N in the plant tissues were determined in 5–10 mg subsamples of finely ground material using an

Fig. 4. Effect of (A) management type and soil compaction on the proportion of N derived from BNF, (B) management type and insect pollination on the proportion of N derived from BNF. Means ± standard error are shown ($n = 31$) (see Appendix A: Table 3). Bars with identical letters are not significantly different at $P < 0.05$ (Tukey HSD).

Elemental Analyzer (Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany) coupled in continuous flow mode to an isotope ratio mass spectrometer (DeltaV, Thermo Fisher Scientific, Bremen, Germany). Average ^{15}N abundances in barley plants were used as reference (Hauggaard-Nielsen, Holdensen, Wulfsohn, & Jensen 2010) for calculating the proportion N derived from BNF by faba bean. The percentage of plant N derived from BNF was determined as:

$$\%N \text{ derived from BNF} = \left(\frac{\delta^{15}N \text{ ref} - \delta^{15}N \text{ faba}}{\delta^{15}N \text{ ref} - B} \right) \times 100$$

The B-value is a measure of isotopic fractionation during N_2-fixation. In this study the B-value used was (-1.33), which is determined for faba bean shoot tissue grown in an N-free medium (100% reliance on N_2-fixation for N acquisition) (López-Bellido, López-Bellido, Redondo, & López-Bellido 2010).

Bumblebee visits

To assess the impact of the treatments on flower visitation by bumblebees, we observed the bumblebees at four occasions between the 6th and 20th of June by following

individual bumblebees and recording which plants they visited (666 observations). Bumblebees were observed between 10:00 and 14:00.

Statistical analyses

We used linear mixed models to test for the effects of management type, soil compaction, and pollination and all interactions on yield components, and the contribution of BNF to nitrogen acquisition of faba bean. Management type, compaction, and pollination were included as fixed factors and block as a random factor. We included all pairwise interactions and selected the best model based on AIC (delta AIC was set to =5) (Burnham & Anderson 2002) using the *dredge* function in package MuMIn (Bartón 2014). The best model was fitted using restricted maximum likelihood (REML). These analyses are presented in Appendix A. A Tukey's post hoc test was used for pairwise comparisons of treatments. We graphically validated the underlying statistical assumptions of linear models. One pot had to be removed from the analysis because no plant emerged. All analyses were performed in the lme4 package (Pinheiro & Bates 2009) using the statistical language R (R Core Team 2012). Seed yield, proportion of N derived from BNF and bumblebee visits were tested for Pearson's correlation coefficient with above- and belowground biomass.

Results

Seed yield

Overall, seed yield was reduced when plants were grown in soil from the ley rotation and under high soil compaction, whereas it was enhanced by insect pollination (Fig. 2). Plants grown in soil from the ley rotation had 18.6% lower seed yield than plants grown in soil from the barley monoculture ($F_{1,66} = 4.56$, $P = 0.04$). Plants grown under high soil compaction had 34.6% lower seed yield than plants grown under low soil compaction ($F_{1,66} = 18.85$, $P < 0.01$). In the presence of pollinators, plants had 38.1% higher seed yield than plants for which pollinators were excluded ($F_{1,66} = 11.03$, $P < 0.01$). There were no significant interactive effects of the treatments on seed yield ($P > 0.23$ in all cases) (see Appendix A: Table 1).

Yield components

Plants grown in soil from the ley rotation produced 21.3% fewer seeds per plant ($F_{1,66} = 5.43$, $P = 0.02$), and had 28.9% lower seed set ($F_{1,67} = 10.76$, $P < 0.01$). High soil compaction reduced the individual seed weight by 32.2% ($F_{1,65} = 14.43$, $P < 0.01$), the number of pods per plant by 19.2% ($F_{1,65} = 5.54$, $P = 0.02$), and tended to reduce the number of seeds per plant ($F_{1,66} = 3.67$, $P = 0.07$). Soil compaction did not influence seed set ($F_{1,62} = 0.60$, $P = 0.44$).

In the presence of pollinators, plants produced 44.3% more seeds per plant ($F_{1,66} = 12.56$, $P < 0.01$), and had 33.4% higher seed set ($F_{1,67} = 15.16$, $P < 0.01$). In the absence of pollinators, plants grown in soil from the ley rotation had 32.4% lower individual seed weight ($F_{1,65} = 4.16$, $P = 0.03$, Fig. 3) and 40.8% fewer pods per plant ($F_{1,65} = 10.17$, $P < 0.01$), while this effect was not found for plants grown in soil from the barley monoculture. There was a positive correlation between seed yield and number of seeds per plant ($r^2 = 0.82$, $F_{1,77} = 152.70$, $P < 0.001$), and number of pods per plant ($r^2 = 0.66$, $F_{1,77} = 60.20$, $P < 0.001$, see Appendix A: Fig. S1). The number of flowers per plant was reduced under high soil compaction for plants grown in soil from the ley rotation, and in the absence of pollinators, plants grown under low compaction produced a greater number of flowers (see Appendix A: Table 4).

Proportion of N acquisition derived from BNF and plant nitrogen concentration

Overall, there were interactive effects between management type and compaction and between management type and pollination on the proportion of N derived from BNF (see Appendix A: Table 3). In soil from the barley monoculture, the proportion of N derived from BNF was reduced for plants grown under high soil compaction, while this effect was not found for plants grown in soil from the ley rotation ($F_{1,21} = 34.17$, $P < 0.01$, Fig. 4A). Nodule number and nodule weight were also reduced for plants grown under high soil compaction by 72% and 70.8% respectively (data not shown). In the presence of pollinators, the proportion of N derived from BNF was lower for plants grown in soil from the barley monoculture compared to plants grown in soil from the ley rotation ($F_{1,21} = 5.63$, $P = 0.04$, Fig. 4B).

Plant N concentration was reduced by 18% for plants grown in soil from the monoculture ($F_{1,26} = 8.25$, $P = 0.01$). Soil total N was reduced by 17% in the soil from the barley monoculture. ($F_{1,67} = 2407.50$, $P < 0.01$). There was a positive correlation between the proportion of N derived from BNF and seed yield ($r^2 = 0.49$, $F_{1,29} = 13.18$, $P = 0.01$). Plants for which the proportion of N derived from BNF was greater were more likely to produce higher seed yield (see Appendix A: Fig. S2).

Growth partitioning

All growth partitioning variables measured were reduced by high soil compaction: total biomass by 18.7% ($F_{1,68} = 40.04$, $P < 0.01$), aboveground biomass by 8.9% ($F_{1,68} = 13.54$, $P < 0.01$), and belowground biomass by 29.2% ($F_{1,68} = 22.68$, $P < 0.01$). High soil compaction reduced the root-to-shoot ratio by 22.0% ($F_{1,68} = 8.46$, $P < 0.01$) and the reproductive-to-vegetative ratio by 25.9% ($F_{1,65} = 9.41$, $P < 0.01$). The reproductive-to-vegetative ratio was also reduced for plants grown in soil from the ley rotation and

was increased for plants grown in the presence of pollinators. Reproductive-to-vegetative ratio was reduced by 22.8% in soil from the ley rotation ($F_{1,65} = 7.07$, $P = 0.01$), and was enhanced by 46.9% when pollinators where present ($F_{1,65} = 15.33$, $P < 0.01$, see Appendix A: Table 2).

There was a positive correlation between aboveground biomass and seed yield ($r^2 = 0.44$, $F_{1,77} = 35.79$, $P < 0.01$). Plants with greater aboveground biomass were more likely to produce higher seed yield. There was no correlation between belowground biomass and seed yield ($r^2 = 0.01$, $F_{1,77} = 0.05$, $P = 1$). There was a positive correlation between the contribution of BNF and aboveground biomass ($r^2 = 0.66$, $F_{1,29} = 30.30$, $P < 0.01$). Plants for which the contribution of BNF was greater were more likely to produce greater aboveground biomass. There was no correlation between contribution of BNF and belowground biomass ($r^2 = 0.09$, $F_{1,29} = 1.15$, $P = 1$, see Appendix A: Fig. S2).

Bumblebee visits to plants and pollination treatment

There was no effect of management type and soil compaction on bumblebee visitation during the experiment (see Appendix A: Table 3). We assume that the open pollination treatment resulted in full pollination.

Discussion

Management factors under intensive agriculture have been associated with yield decline and stagnation (Foley et al. 2005). We have demonstrated that plant responses to these factors can affect pollination benefits on seed size, but this did not affect the seed yield. We showed that combination of soil management affected the contribution of BNF to N acquired by the plant but, again, this effect was not reflected at the seed yield level.

Insect pollination contributed 38.1% to seed yield in our study, which is in accordance with a general estimate for the contribution of pollination of 36% to yield of faba bean (Free 1993). Previous exclusion experiments have found that faba bean produced fewer pods, fewer seeds per pod, and lower yield in absence of pollination (Poulsen 1975; Free & Williams 1976; Stoddard & Bond 1987; Suso, Moreno, Mondragao-Rodrigues, & Cubero 1996), and our results largely confirm this.

We found no interactive effects between management type, soil compaction, and insect pollination on seed yield of faba bean. However, in the absence of pollinators individual seed weight and number of pods per plant were reduced in soil from the ley rotation. This suggests that the effect found in the soil from the ley rotation predominantly affects fruits generated via self-pollination. Self-pollination has been shown to lead to higher fruit abortion than cross-pollination (Pflugshaupt, Kollmann, Fischer, & Roy 2002) and resource

limitation has also been shown to increase the effect of self-pollination on fruit abortion in an annual legume (Martin & Lee 1993). Increased individual seed weight and pods per plant were found for faba beans exposed to pollinators (Suso et al. 1996). The positive correlation between seed yield and number of seed per plant suggest that plants grown in soil from the ley rotation compensated the small seed produced in the absence of pollinators by increasing seed number so that interactive effects were not detected at the seed yield level. Variation in seed size may affect the capacity of seedling to establish in the field (Marshall 1986). This could have important implications for seed growers where plants grown under stress benefit from pollinators for seed quality.

Contrary to our expectations, a reduction in seed yield, number of seeds per plant and seed set occurred in soil from the ley rotation, and not in the barley monoculture. One explanation could be that plants grown in the ley soil were limited in potassium. Our analysis of the soil characteristics showed that soil from the ley rotation contained 50% less potassium than the soil from the monoculture (Table 1). Leys are known for high potassium consumption, and a great ability to exploit potassium resources in the soil (Lanyon & Smith 1985). This means that although leys are effective in maintaining high levels of soil organic carbon and associated N (Kremen & Miles 2012), other nutrients can become limiting for the plant and impair its expected effect on yield. Potassium has been shown to stimulate flower induction in olives (Fabbri & Benelli 2000) suggesting that flower production was limited for plants grown in the ley rotation.

The lowest proportion of N derived from BNF found in the highly compacted monoculture suggests that soil compaction exacerbates the negative effect of monoculture on BNF. The intermediate proportion of N derived from BNF found in the highly compacted soil from the ley rotation suggests that the properties of the soil from the ley rotation allow it to mitigate the negative effect of high soil compaction. Increases in soil organic matter are known to increase soil elasticity and resistance to deformation thereby reducing the effect of compaction (Soane 1990). The negative effect of high soil compaction found in soil from the barley monoculture was not mirrored at the seed yield level. However, we found that the proportion of N derived from BNF was positively associated with seed yield. The high proportion of N derived from BNF in the low-compaction monoculture is related to the lower soil N content of the monoculture soil, which enhances N fixation in faba bean (Sprent & De Faria 1988). High levels of soil N have been shown to reduce the amount of N_2 fixed by legumes (Peoples, Ladha, & Herridge 1995; Jensen & Schjoerring 1997) while low compaction has been shown to favor nodulation and N fixation (Buttery et al. 1998). We found that plant N concentration was reduced for plants grown in soil from the barley monoculture. This can partly be explained by the low N content of the monoculture soil. Grass/ley mixtures have been shown to contribute sufficient N to lower N fertilization requirement to subsequent crops (Johnston 1990; Nevens & Reheul 2002). The low plant N concentration found for plants grown in soil from the barley monoculture also implies that the benefits as green manure of field bean grown under these soil conditions would be lower.

Furthermore, we found an interactive effect of management type and pollination on the proportion of N derived from BNF which suggests that enhanced seed yield by pollination increases plant demand for N. Nitrogen fixation in faba bean peaks between flowering and grain filling (Silsbury 1990). The relationship between pollinators and rhizobia is unclear and we have no leads in the literature to explain mechanisms for this pattern. Further studies are clearly needed to identify how insect pollination moderates N fixation.

Recent studies have found interactive effects between nutrients and pollination, but they show contrasting directions. In sunflower, the benefit of pollination on seed set and yield was only observed when plants were grown in soils with high, but not low, fertility (Tamburini, Berti, Morari, & Marini 2015). In oilseed rape, the benefit of pollination on yield was maintained when nitrogen fertilization was removed (Marini et al. 2015). In almond, nutrient management did not affect the contribution of insect pollination to yield and yield components; although yellow leaves observed in the pollinated treatment where no nutrients were added suggest that nutrients might have been remobilized from the leaves to the fruits (Klein et al. 2015). In faba bean we found no interaction between soil management and pollination at the yield level. It is possible that compensation for nutrient limitation by pollination, as observed in oilseed rape, does not occur because faba bean is a legume and derives extra N from BNF. This is supported by the positive correlation between high yield and high proportion of N derived from BNF. Various studies have shown yield of faba bean to be correlated with BNF (Hauser 1987; Köpke 1996; Schulz, Keatinge, & Wells 1999). Also aboveground biomass was positively correlated with yield. This suggests that seed production was directly supported by photosynthesis and BNF. Further studies are needed to investigate the contribution of pollination to yield in crop species with different N requirements.

We acknowledge a number of caveats to the findings presented in this study, so caution must be exercised when generalizing these results. First, the experiment was conducted under greenhouse conditions and seed yield observed was relatively low. However, some interesting impacts on seed yield were observed that deserve further study under more realistic field conditions. Second, the contrast between management type barley monoculture and ley rotation cannot be generalized to represent differences between actual monocultures and crop rotations since, for that, soil from a population of monocultures and of rotations would need to be sampled. Thus, future studies should examine plant response from different fields with same management history.

Conclusion

In conclusion, soil compaction and monoculture diminish the contribution of BNF to N acquisition of faba bean. If applicable to field conditions, these findings are clearly relevant for agriculture. By managing soils such that soil quality is deteriorated, farmers reduce legumes' ability to supply N, potentially increasing dependency on mineral N inputs and at the same time reducing key ecosystem services delivered by legumes in the agroecosystem.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.baae.2016.07.001.

References

Ball, B. C., Bingham, I., Rees, R. M., Watson, C. A., & Litterick, A. (2005). The role of crop rotations in determining soil structure and crop growth conditions. *Canadian Journal of Soil Science*, *85*(5), 557–577.

Barber, N. A., & Gorden, N. L. S. (2014). How do belowground organisms influence plant–pollinator interactions? *Journal of Plant Ecology*, *8*, 1–11.

Bartón, K. (2014). *MuMIn: Multi-model inference. R package (version 1.7.7)*.

Bennett, A. J., Bending, G. D., Chandler, D., Hilton, S., & Mills, P. (2012). Meeting the demand for crop production: The challenge of yield decline in crops grown in short rotations. *Biological Reviews*, *87*, 52–71.

Bommarco, R., Kleijn, D., & Potts, S. G. (2013). Ecological intensification: Harnessing ecosystem services for food security. *Trends in Ecology and Evolution*, *28*, 230–238.

Boreux, V., Kushalappa, C. G., Vaast, P., & Ghazoul, J. (2013). Interactive effects among ecosystem services and management practices on crop production: Pollination in coffee agroforestry systems. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(21), 8387–8392.

Bos, M. M., Veddeler, D., Bogdanski, A. K., Klein, A. M., Tscharntke, T., Steffan-Dewenter, S., et al. (2007). Caveats to quantifying ecosystem services: Fruit abortion blurs benefits from crop pollination. *Ecological Applications*, *17*(6), 1841–1849.

Brereton, J. C., McGowan, M., & Dawkins, T. C. K. (1986). The relative sensitivity of spring barley, spring field beans and sugar beet crops to soil compaction. *Field Crops Research*, *13*, 223–237.

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach*. Springer Science and Business Media.

Buttery, B. R., Tan, C. S., Drury, C. F., Park, S. J., Armstrong, R. J., & Park, K. Y. (1998). The effects of soil compaction, soil moisture and soil type on growth and nodulation of soybean and common bean. *Canadian Journal of Plant Science*, *78*(4), 571–576.

Cunningham, S. A., & Le Feuvre, D. (2013). Significant yield benefits from honeybee pollination of faba bean (*Vicia faba*) assessed at field scale. *Field Crops Research*, *149*, 269–275.

Cuvardic, M., Tveitnes, S., Krogstad, T., & Lombnæs, P. (2004). Long-term effects of crop rotation and different fertilization systems on soil fertility and productivity. *Acta Agriculturae Scandinavica, Section B – Soil and Plant Science*, *54*(4), 193–201.

Egnér, H. A. N. S., Riehm, H., & Domingo, W. R. (1960). Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor-und Kaliumbestimmung. *Kungliga Lantbrukshögskolans Annaler*, *26*, 199–215.

Fabbri, A., & Benelli, C. (2000). Flower bud induction and differentiation in olive. *Journal of Horticultural Science and Biotechnology*, *75*(2), 131–141.

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., et al. (2005). Global consequences of land use. *Science*, *309*, 570–574.

Free, J. B. (1993). *Insect pollination of crops*. London: Academic Press., 684 pp.

Free, J. B., & Williams, I. H. (1976). Pollination as a factor limiting the yield of field beans (*Vicia faba* L.). *The Journal of Agricultural Science*, *87*(2), 395–399.

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., et al. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*, *339*, 1608–1611.

Grover, K. K., Karsten, H. D., & Roth, G. W. (2009). Corn grain yields and yield stability in four long-term cropping systems. *Agronomy Journal*, *101*(4), 940–946.

Gwata, E., Wofford, D., Pfahler, P., & Boote, K. (2003). Pollen morphology and in vitro germination characteristics of nodulating and nonnodulating soybean (*Glycine max* L.) genotypes. *Theoretical and Applied Genetics*, *106*(5), 837–839.

Hamza, M. A., & Anderson, W. K. (2005). Soil compaction in cropping systems: A review of the nature, causes and possible solutions. *Soil and Tillage Research*, *82*, 121–145.

Hauggaard-Nielsen, H., Holdensen, L., Wulfsohn, D., & Jensen, E. S. (2010). Spatial variation of N_2-fixation in field pea (*Pisum sativum* L.) at the field scale determined by the ^{15}N natural abundance method. *Plant and Soil*, *327*, 167–184.

Hauser, S. (1987). *Schätzung der symbiotisch fixierten Stickstoffmenge von Ackerbohnen (Vicia faba L.) mit erweiterten Differenzmethoden* Diss. Agr. Germany: University of Göttingen.

Jensen, E. S., Peoples, M. B., & Hauggaard-Nielsen, H. (2010). Faba bean in cropping systems. *Field Crops Research*, *115*(3), 203–216.

Jensen, H., & Schjoerring, J. K. (1997). Interactions between white clover and ryegrass under contrasting nitrogen availability: N_2 fixation, N fertilizer recovery, N transfer and water use efficiency. *Plant and Soil*, *197*(2), 187–199.

Johnston, A. E. (1990). Soil fertility and soil organic matter. In W. A. Wilson (Ed.), *Advances in soil organic matter research* (pp. 299–314). Cambridge: The Royal Society of Chemistry.

Kearns, C. A., & Inouye, D. W. (1993). *Techniques for pollination biologists*. University Press of Colorado.

Klein, A. M., Hendrix, S. D., Clough, Y., Scofield, A., & Kremen, C. (2015). Interacting effects of pollination, water and nutrients on fruit tree performance. *Plant Biology*, *17*, 201–208.

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., et al. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society of London B: Biological Sciences*, *274*, 303–313.

Kremen, C., & Miles, A. (2012). Ecosystem services in biologically diversified versus conventional farming systems: Benefits, externalities, and trade-offs. *Ecology and Society*, *17*(4), 40.

Köpke, U. (1996). *Symbiotische Stickstoff-Fixierung und Vorfruchtwirkung von Ackerbohnen (Vicia faba L.)* Habilitation thesis. Germany: University of Göttingen.

Lancashire, P. D., Bleiholder, H., Boom, T. V. D., Langelüddeke, P., Stauss, R., Weber, E., et al. (1991). A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology*, *119*(3), 561–601.

Lanyon, L. E., & Smith, F. W. (1985). *Potassium nutrition of alfalfa and other forage legumes: Temperate and tropical*.

Lin, M., & Huybers, P. (2012). Reckoning wheat yield trends. *Environmental Research Letters*, *7*(2), 024016.

Lipiec, J., & Hatano, R. (2003). Quantification of compaction effects on soil physical properties and crop growth. *Geoderma*, *116*(1), 107–136.

López-Bellido, F. J., López-Bellido, R. J., Redondo, R., & López-Bellido, L. (2010). B value and isotopic fractionation in N_2 fixation by chickpea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.). *Plant and Soil*, *337*, 425–434.

Löfkvist, J., Whalley, W. R., & Clark, L. J. (2005). A rapid screening method for good root-penetration ability: Comparison of species with very different root morphology. *Acta Agriculturae Scandinavica, Section B – Soil and Plant Science*, *55*, 120–124.

Löfkvist, J., Rydberg, T., & Svantesson, U. (2000). A method for screening the ability of plant roots to penetrate soil and increase hydraulic conductivity. In *Proceedings of the 15th conference of the international soil tillage research organization*.

Lundin, O., Smith, H. G., Rundlöf, M., & Bommarco, R. (2013). When ecosystem services interact: Crop pollination benefits depend on the level of pest control. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*(1753), 20122243.

Marini, L., Tamburini, G., Petrucco-Toffolo, E., Lindström, S. A. M., Zanetti, F., Mosca, G., et al. (2015). Identifying options for replacing management inputs with ecosystem services. *Agriculture, Ecosystems and Environment*, *207*, 61–66.

Marshall, D. L. (1986). Effect of seed size on seedling success in three species of Sesbania (Fabaceae). *American Journal of Botany*, 457–464.

Martin, M. E., & Lee, T. D. (1993). Self pollination and resource availability affect ovule abortion in Cassia fasciculate (Caesalpiniaceae). *Oecologia*, *94*, 503–509.

Nevens, F., & Reheul, D. (2002). The nitrogen-and non-nitrogen-contribution effect of ploughed grass leys on the following arable forage crops: Determination and optimum use. *European Journal of Agronomy*, *16*(1), 57–74.

Peoples, M. B., Brockwell, J., Herridge, D. F., Rochester, I. J., Alves, B. J. R., Urquiaga, S., et al. (2009). The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis*, *48*, 1–17.

Peoples, M. B., Ladha, J. K., & Herridge, D. F. (1995). Enhancing legume N_2 fixation through plant and soil management. In *Management of biological nitrogen fixation for the development of more productive and sustainable agricultural systems*. pp. 83–101. Netherlands: Springer.

Pflugshaupt, K., Kollmann, J., Fischer, M., & Roy, B. (2002). Pollen quantity and quality affect fruit abortion in small populations of a rare freshly-fruited shrub. *Basic and Applied Ecology*, *3*, 319–327.

Pinheiro, J. C., & Bates, D. M. (2009). *Mixed effects models in S and S-Plus*. New York: Springer.

Poulsen, M. H. (1975). Pollination seed setting, cross-fertilisation and in breeding in *Vicia faba* L. *Zeitschrift für Pflanzenzüchtung*.

Puentes, A., Bazely, D. R., & Huss-Danell, K. (2007). Endophytic fungi in *Festuca pratensis* grown in Swedish agricultural grasslands with different managements. *Symbiosis*, *44*(1–3), 121–126.

R Core Team. (2012). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0. http://www.R-project.org/

Ray, D. K., Ramankutty, N., Mueller, N. D., West, P. C., & Foley, J. A. (2012). Recent patterns of crop yield growth and stagnation. *Nature Communications*, *3*, 1293.

Scheu, S. (2001). Plants and generalist predators as links between the below-ground and above-ground system. *Basic and Applied Ecology*, *2*, 3–13.

Schulz, S., Keatinge, J. D. H., & Wells, G. J. (1999). Productivity and residual effects of legumes in rice-based cropping systems in a warm-temperate environment. I. Legume biomass production and N fixation. *Field Crops Research*, *61*(1), 23–35.

Silsbury, J. H. (1990). Growth, nitrogen accumulation and partitioning, and N_2 fixation in faba bean (*Vicia faba* cv. Fiord) and pea (*Pisum sativum* cv. Early Dun). *Field Crops Research*, *24*(3–4), 173–188.

Shearer, G., & Kohl, D. H. (1986). N_2-fixation in field settings: Estimations based on natural ^{15}N abundance. *Australian Journal of Plant Physiology*, *13*, 699–756.

Soane, B. D. (1990). The role of organic matter in soil compatibility: A review of some practical aspects. *Soil and Tillage Research*, *16*(1), 179–201.

Sprent, J. I., & De Faria, S. M. (1988). Mechanisms of infection of plants by nitrogen fixing organisms. *Plant and Soil*, *110*(2), 157–165.

Stoddard, F. L., & Bond, D. A. (1987). The pollination requirements of the faba bean. *Bee World*, *68*(3), 144–152.

Suso, M. J., Moreno, M. T., Mondragao-Rodrigues, F., & Cubero, J. I. (1996). Reproductive biology of *Vicia faba*: Role of pollination conditions. *Field Crops Research*, *46*(1), 81–91.

Tamburini, G., Berti, A., Morari, F., & Marini, L. (2015). Degradation of soil fertility can cancel pollination benefits in agricultural crops. *Oecologia*, http://dx.doi.org/10.1007/s00442-015-3493-1

van Eekeren, N., Bommelé, L., Bloem, J., Schouten, T., Rutgers, M., de Goede, R., et al. (2008). Soil biological quality after 36 years of ley-arable cropping, permanent grassland and permanent arable cropping. *Applied Soil Ecology*, *40*(3), 432–446.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., Van Der Putten, W. H., & Wall, D. H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, *304*(5677), 1629–1633.