PROTEIN 2FOOD

Project acronym: PROTEIN2FOOD

Project No.: 635727

H2020-SFS-2014-2015/H2020-SFS-2014-2

Start date of project: March 2015

Duration: 5 years

Deliverable reference number and title

D1.7 Effects on soil fertility, biodiversity, P-mobilization and N-fixation

Date: 28.02.2019

Organisation name of lead for this deliverable:

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Project co-funded by the European Commission within the Horizon 2020 Programme		
Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635727.

0. Summary

In this study, the effects of grain legumes on nitrogen fixation, phosphorus-mobilization, soil fertility and biodiversity were investigated. The extended nitrogen difference method was used to measure nitrogen fixation by faba bean and white lupin, using spring wheat as a reference crop. Soil nitrogen modelling with the NDICEA model was used as an additional tool to estimate nitrogen fixation. Both the extended nitrogen difference method and NDICEA modelling resulted in relatively low estimates of the amount of nitrogen fixated: 122 kg N/ha (extended difference method) versus 120 kg N/ha (uncalibrated NDICEA model) by faba bean. The respective amounts by white lupin were 80 kg N/ha versus 75 kg N/ha. Calibration of the NDICEA model proved difficult because of the very dry weather conditions during 2018, which further decreased estimates of N-fixation.

Soil phosphorus mobilization was measured by calculating mineral balances for different plant parts of faba bean, white lupin and spring wheat. Low amounts of phosphorus were returned by crop residues to the soil after harvest. Furthermore, no differences could be found in plant-available phosphorus levels (Pw and P-PAE) or longer-term available phosphorus (P-AL) after harvest. These experiments were done based on field trials with a variety of protein crops (blue and white lupin, quinoa, faba bean, soybean and buckwheat), and compared with spring wheat as a reference crop.

Furthermore, the effect of grain legumes on the abundance and diversity of pollinators was measured in faba bean and white and blue lupin, as an indicator on local biodiversity. It was found that both small-scale landscape elements and the presence of nature areas in the vicinity of farmland are important aspects in maintaining an abundant and diverse pollinator community.

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1. Introduction and objectives

In this deliverable, the effects of grain legumes on soil fertility, P-mobilization, N-fixation and biodiversity were investigated. The background and main objectives of the deliverable are described and introduced below.

1.1 Effect of grain legumes on N-fixation, soil fertility and P-mobilization

N-fixation

The inclusion of grain legumes in arable crop rotations can have positive effects on soil fertility. Grain legumes fixate atmospheric nitrogen during the growing season, and enhance nitrogen availability for the subsequent crop. Calculations of global contributions of biological nitrogen fixation are thought to be in the order of 20-22 million tonnes of nitrogen per year (Peoples, 2009). This nitrogen will partly be consumed as grains, pods or tubers, and partly be returned as a renewable nitrogen source, which enhances soil fertility. Different methods have been employed to estimate the nitrogen fixation by leguminous crops, all with different limitations. The acetylene reduction assay (ARA) has been widely used in the past, but it depends on conversion factors to translate results into nitrogenase activity. The use of the ^{15}N dilution method is based on the assumption that both the legume and the reference crop will use soil nitrogen at the same $^{15}N/^{14}N$ ratio. This condition is difficult to meet, as rooting patterns and depths of legumes and reference crops differ (Hauser, 1990). The initial N-difference method was used only plant shoots, but it was developed into an extended N-difference method, in which all the plant parts were taken into account, including litter, roots and nodules. German research on faba bean showed comparable results of the ^{15}N dilution method and the extended N-difference method (Hauser, 1990). Because of the lower costs of materials and laboratory analysis of the N-difference method, we have used the method in the PROTEIN2FOOD trials to estimate nitrogen fixation in the 2018 trials in Klazienaveen, The Netherlands.

Soil fertility

Additionally, we have used N-modeling with the NDICEA model to gain insight in both nitrogen fixation and effects on soil-fertility. Modelling nitrogen and carbon dynamics in soils can be used as a method to determine the effect of grain legumes on long-term soil fertility. In addition, application-oriented models can serve as decision-making tools for farmers and consultants. The NDICEA model entails qualities of both a rigorous scientific background, as well as a design aimed at practical application (Van der Burgt et al, 2016). NDICEA is built as a target-oriented model, where crop yields are used as input data. The model has been developed for Dutch conditions, but has also been successfully applied in other countries. Farmers can use the model as a tool to gain insight into the effects of crop rotation and fertilization on soil fertility, nitrogen dynamics, as well as long-term soil organic matter development (www.ndicea.nl). The model has been extensively used and tested with legumes, especially clover and alfalfa, but until now, very little knowledge or experience had been gained of grain legumes such as faba bean and lupin (Van der Burgt,

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pers.com). Faba bean has already been included into the model, but lupin not yet. However, with the data we have gathered in this study, lupin can – in a preliminary form - be included in the model.

Phosphorus mobilization by grain legumes

Crops differ in their capacity to mobilize phosphorus (P) from soil. Studies have shown that grain legumes are capable of a better phosphorus uptake. Subsequent crops, such as grains, grown in the same area profit from the higher phosphorus mobilization caused by legumes. It has been hypothesized that this could be either due to an excess of root exudates released during the growth of legumes, or due to the breakdown of P-containing crop residues during the following growing season. Nuruzzaman et al (2005) found in pot experiments that lupin, and faba bean produced substantial amounts of rhizosphere carboxylates, whilst negligible amounts were found in the wheat rhizosphere. Furthermore, it was found that wheat grew better in pots where legumes had been grown previously, compared to pots where wheat had been grown or pots that had been unplanted. In the Nuruzzaman study, when only legume roots were incorporated in pots, wheat growth was not enhanced further. Generally, legumes are thought to be more efficient in extracting soil P than cereals, but results are not consistent. A study with faba bean, chickpea, wheat and canola on both acid and calcareous soils showed no difference in the capacity to access insoluble P-pools among the different crops, with the exception of depletion of one of the insoluble fractions by faba bean on acid soil (Rose et al, 2010). Intercropping of cereals and legumes also leads to increased mobilization of P, compared to monoculture cereals on phosphorus-deficient soils. Examples were found in experiments on maize and faba bean (Li et al, 2007); and chickpea, field pea and lupin combined with wheat (Espinosa, 2014). White lupin has the ability to form cluster roots with special characteristics that allow phosphorus uptake even under circumstances with very poor P availability (Cheng et al, 2011).

1.2 Effect of grain legumes on biodiversity

The introduction of grain legumes in crop rotations may affect biodiversity in several ways. In the past, crops such as faba bean were grown in large areas of Europe. In the Netherlands for example, the disappearance of grain legumes in cropping systems coincided with the disappearance of other flowering crops, like flax and caraway seed. Flowering crops might play an important role in maintenance of wild pollinator communities, by providing nectar and pollen. On the other hand, faba bean is also known to be dependent on wild pollinators, especially bumblebees, for obtaining good yields. In a comparison of open-pollinated versus self-pollinated faba bean, researchers found a 185% yield increase due to the presence of pollinators (Nayak et al, 2015). For farmers, it is therefore important to know which pollinators are attracted to specific grain legumes, in order to be able to create optimal environment, both in terms of nesting facilities and food sources, for the pollinators. Therefore, the objective of this study was to investigate the presence of wild pollinators in relation to different grain legumes and environments in the Netherlands.

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2. Activities for solving the tasks

2.1 Effect of grain legumes on soil fertility, N-fixation and P-mobilization

N-fixation

In our research, the extended N difference method was used to estimate biologically nitrogen fixation by faba bean and lupin (Neugschwandtner et al, 2015). Field experiments were performed in 2018 in Klazienaveen, in the Netherlands. White lupin (Boros), faba bean (Fuego), spring wheat (Lavett) and fallow plots were situated in a completely randomized block design with three replications. Spring wheat was chosen as a reference crop, as unfertilized wheat has a very efficient nitrogen uptake and is able to deplete all available nitrogen in the soil. Thus, an estimation of the maximum soil nitrogen uptake by legumes on a specific soil type could be obtained, as well as an estimation of the minimum amount of nitrogen fixated. The experimental fields were located on a sandy soil with high organic matter content (former peat soil). Crops were sown on April 9, and harvested on August 9, 2018. At the end of the growing season, crops were harvested and separated in four different fractions (grain, shaff/pods, straw and root/stubble). Fresh and dry weight of the fractions was determined, and subsamples were analysed for nutrient contents. Mineral nitrogen contents were determined in the soil shortly after harvest.

Soil fertility

To calibrate the NDICEA model, data on air temperature, precipitation and soil moisture contents were collected from a small weather station at the field location in Klazienaveen. Data on evapotranspiration (ET0) were gathered from the nearest KNMI station in Hoogeveen (35 km distance), and applied by the Makkink formula, using temperature and solar radiation (Makkink, 1957). The model uses average daily values for all these parameters. Three times during the growing season, soil mineral nitrogen was measured in the topsoil of all the replicate plots. Soil mineral nitrogen measurements in fallow and wheat plots were used to calibrate the model. Calibration parameters were used to facilitate modelling of faba bean and lupin. In order to estimate the size of the different organic matter pools in the soil, the crop and fertilization history of the field was entered into the model, starting from two years prior to the current growing season. For years 2016 and 2017, precipitation data of the nearest KNMI rain tower (located in Klazienaveen) were combined with temperature and ET0 values from the larger KNMI weather station in Hoogeveen.

P-mobilization

In our research, we used two different approaches to assess P-mobilization by leguminous crops. The first approach is based on measurements of mobile, plant-available P-fractions in the soil, before and after cultivation of leguminous crops. The second approach applies mineral balances to assess the amount of phosphorus released and available through crop

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residues at the end of the growing season. For this approach, the method of the 2018 field trials for nitrogen fixation in Klazienaveen was used, calculating mineral balances for phosphorus for white lupin, faba bean and spring wheat.

In the second approach, phosphorus fractions were measured in the soil before and after the field experiments. In 2015, field trials were carried out at two locations: Holten and Klazienaveen, to investigate changes in available phosphorus in soil. At both locations, white and blue lupin were cultivated, and spring wheat and fallow were included as reference crop and control treatment. In Klazienaveen, faba bean was included in the trials as well. In Klazienaveen, trials were located on a sandy, former peat soil with 9.9% organic matter and pH 5.1. In Holten, experiments were carried out on a sandy soil. The experimental design was a completely randomized block design with three replicates at both locations. In 2016, field trials were carried out at a second field location in Klazienaveen, with white and blue lupin, faba bean, soybean, quinoa and buckwheat. Spring wheat and fallow were included as reference crops. The soil is a sandy, former peat soil, with 5.8% organic matter and pH 5.2. Experimental design was again a completely randomized block design with three replicates. Three phosphorus fractions were measured in the topsoil (0-25 cm) using three methods of P-PAE, Pw and P-AL. Details on the different methods of phosphorus measurement can be found in appendix 8.2. Before the start of the growing season, phosphorus fractions were measured in a mixed sample, and at the end of the growing season, fractions were measured separately in each plot.

2.2 Effect of grain legumes on biodiversity

The effect of grain legumes on pollinators (honey bees, wild bees, bumble bees, hoverflies and remaining groups) was determined at three experimental locations in The Netherlands (Klazienaveen, Bellingwolde and Oostwold). In Oostwold, fields with faba bean, in Bellingwolde, fields with blue lupin and faba bean, and in Klazienaveen, fields with blue and white lupin and spring faba bean were visited twice during bloom season. In each crop, an inventory was made by walking 6 transects (25 m. length, 10 min/transect), and all flower-visiting pollinators were recorded. When species identification in the field was difficult, bees were collected and identified in the laboratory. Data collection was overseen by entomologists Peter de Boer and Jeroen Breidenbach (Faunax). Additional historical information on pollen diet and behavior on pollinators visiting flowers of lupin and faba bean was provided by Jeroen Scheper (Alterra) and Menno Reemer (EIS-Netherlands).

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3. Results and discussion

3.1 Soil Fertility, N-fixation and P-mobilization

N-fixation

Crop development in the 2018 field trials was affected by several factors. All crops germinated well, but spring wheat suffered from mechanical weed suppression, which was carried out perpendicular to the sowing direction, as this is the preferential direction for the leguminous crops in the trial. Development of white lupin during the growing season suffered from the early onset of fungal diseases, which came to a halt in the warm and dry weather conditions (see Figure 1 and Table 1). From April onward, average monthly temperatures were higher than normal, and especially rainfall was very low. Spring-sown faba bean suffered the most from these extremely dry weather conditions. Harvest dates were earlier than on average, and all crops had been harvested on August 9. Overall yields were reasonable for white lupin (2.4 ton/ha, 15% moisture), moderate for faba bean (4.1 ton/ha, 15% moisture), and low for spring wheat (2.5 ton/ha, 15% moisture).

Table 1. Average monthly temperatures KNMI weather station Hoogeveen in 2018 (T 2018), and long-term average (30-year period from 1981-2010) (T normal).

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
T 2018	4.6	-0.3	3.9	11.7	16.5	16.8	19.8	18.0	14.4	11.4	6.0	5.6
T normal	3.1	3.3	6.2	9.2	13.1	15.6	17.9	17.5	14.5	10.7	6.7	3.7

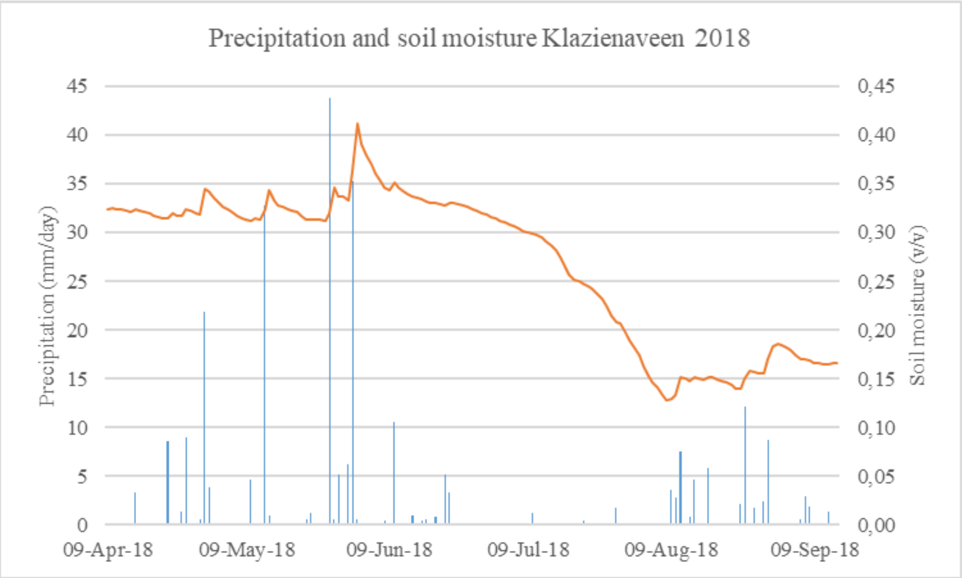

Figure 1. Precipitation and soil moisture during the growing season of lupin, faba bean and spring wheat in Klazienaveen trials in 2018.

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Macro- and micro nutrient contents of lupin, faba bean and spring wheat were measured and the results are presented in Table 6 and 7 in the appendix. The mineral balance for nitrogen is shown for white lupin, faba bean and spring wheat in Figure 2. The treatment with unfertilized spring wheat was included, as it provides information on the maximum amount of mineral nitrogen, which can be obtained from the soil under specific local conditions of soil type and climate. Because of the extended rooting system, spring wheat is a very efficient crop in terms of soil mineral nitrogen use. In turn, the minimum amount of nitrogen fixation by the two leguminous crops (white lupin and faba bean), can be calculated based on the assumption that spring wheat shows the maximum amount of mineral nitrogen being released from the soil (provided there were no other constraints to spring wheat growth). The average nitrogen uptake of spring wheat was 75 kg N/ha. The total crop nutrient contents of white lupin and faba bean were 154 and 197 kg N/ha, respectively. From these mineral balances it is estimated that white lupin fixated at least 80 kg N/ha and faba bean 122 kg N/ha. Differences between soil mineral nitrogen at the end of the growing season were very small between the three crops. In spring wheat, 15 kg/ha soil mineral N was measured 12 days after harvest; in white lupin it was 14 kg/ha, and in faba bean 20 kg/ha.

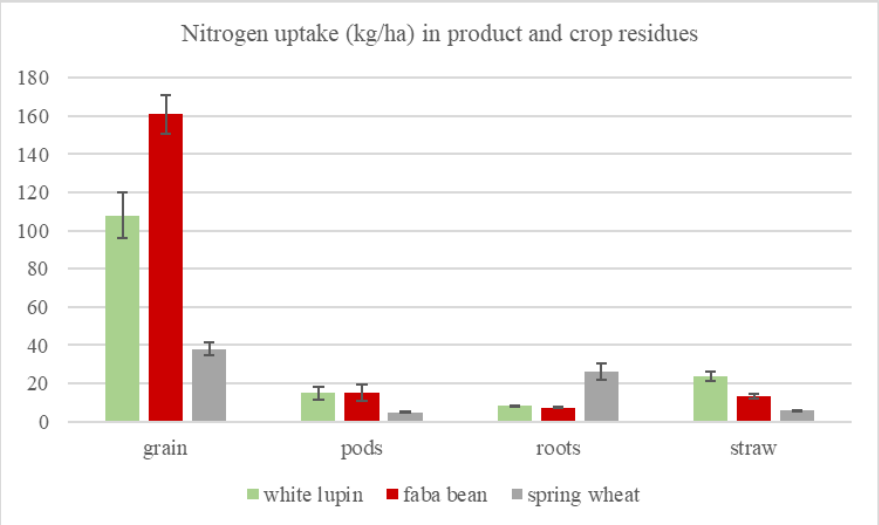

Figure 2. Nitrogen contents of harvested product and crop residues in kg/ha for white lupin, faba bean and spring wheat.

Discussion

The results we obtained with the extended difference method could be compared with previous pot and field experiments by other authors. Nitrogenase experiments by Van Mil (1980) show differences between the actual and potential nitrogen fixation of faba bean, where actual nitrogen fixation equaled 291 mg N_2/plant, and potential nitrogen fixation 416 mg N_2/plant. Translating the pot trial results of Van Mil to a field situation, this would result in amounts of 87 kg N/ha (actual) and 125 kg N/ha (potential) nitrogen fixation, assuming

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a plant density of 30 plants/m^2. The Van Mil data might be difficult to translate to nitrogen fixation levels of current faba bean varieties, but the differences between actual and potential fixation are important to note. Van Mil found that actual nitrogen fixation is negatively affected by high amounts of inorganic nitrogen available in the soil. This implicates that depending on the soil type, crop rotation, soil organic matter pools and climatic conditions, the amount of nitrogen fixated will vary from year to year. In order to provide insight in the effects of mineral nitrogen on nitrogen fixation, we have used NDICEA modelling as an additional tool.

Hauser (1990) found in a comparable study with the simple nitrogen difference method (based only on above-ground shoots) that nitrogen fixation levels in faba bean ranged from 110 to 271 kg N/ha. Using the extended method, he estimated nitrogen fixation ranging from 142 to 330 kg N/ha (Hauser, 1990). Litter, which was collected every 14 days in the 'extended' study of Hauser, contributed with 25-40 kg N/ha. In a more recent study of two year winter faba bean cultivation in Austria, the authors estimated nitrogen fixation levels ranging from 32-135 kg N/ha, when only taking into account the above ground dry matter, and total N fixation ranging from 63-219 kg/ha, when including the nitrogen left into the soil. Yields of winter faba bean in this study ranged between 1.7 and 4.5 tonnes/ha in the two years studied (Neugschwandtner et al, 2015). In summary, nitrogen fixation levels in our study were lower than expected, compared to the moderate yield of faba bean (4.1 tonnes/ha). The susceptibility of faba bean for the very dry season and the (root) disease in lupin might have affected the results.

Nitrogen modelling and effect on soil fertility

For nitrogen modelling in NDICEA, field history (comprising crops, yields, green manures and fertilizers) is important input data. In the Klazienaveen experimental field, potatoes (2016) and oats (2017) were cultivated in the preceding years. Together with input data on (organic) fertilizers, NDICEA estimates the dimensions of three fractions of the soil organic matter pool. NDICEA is a target-oriented model. Measured data on yield and mineral contents of grain, crop residues and roots were used as input data. Measured soil mineral nitrogen in faba bean, white lupin, spring wheat and fallow was used to calibrate the model.

Nitrogen fixation in the NDICEA model is calculated based on the potential fixation rate (in kg/ha/day), and with a threshold inorganic nitrogen amount in the topsoil thr_{fix} (kg N/ha) above which fixation is negatively affected. When nitrogen levels in soil exceed the threshold level, potential fixation is reduced in proportion to the amount of inorganic nitrogen in the topsoil, up to a level of twice the value of thr_{fix} (Van der Burgt et al, 2006). Default values of thr_{fix} have been set at 15 kg N/ha for faba bean. This threshold valued is related to several factors such as soil moisture content, and possibly soil structure and root health. The final value of this factor is found by calibration, and experience has shown that for clover and alfalfa, a thr_{fix} of 5 kg N/ha is more appropriate. For pea, a higher value of

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thr$_{fix}$ seems appropriate, but for lupin and faba bean this is unknown (Van der Burgt, pers.com.).

Modeling results without calibration

Soil moisture measurements revealed that initially the modelling of pF values (soil moisture tension) deviated considerably from the field situation, especially in the last part of the growing season, when soil conditions became very dry. The model predicted sufficient available moisture, while field measurements revealed a serious lack of moisture. Therefore, soil mineral-N levels predicted by the model were higher than those found in the field. Without calibration, and with soil-mineral nitrogen thresholds set at 15 kg N/ha, the model estimated an amount of 120 kg N/ha fixated by Faba bean, and 75 kg N/ha by Lupin, These estimates are well within the range of the calculated amounts of 122 kg N/ha and 80 kg N/ha, by the extended difference method.

Modeling results with calibration

When calibrating the NDICEA model with the help of the mineral nitrogen measured in the topsoil, the measured values for N-min in the soil and the modelled values are closer to each other. However, in order to lower the modelled amount of nitrogen compared to the uncalibrated model, the model assumes a higher nitrogen uptake from the soil by the leguminous crops. The threshold values for fixation are in the calibration of both Faba bean and Lupin lowered to 6 kg N/ha. Below this amount of soil mineral N, the model assumes 100% nitrogen fixation. Above the amount of 2 * thr$_{fix}$, or 12 kg N/ha, the model assumes that no nitrogen is fixated at all. This leads to an estimation of the model of only 45 kg N fixated by Faba bean, and 9 kg N fixated by Lupin. Soil mineral nitrogen levels, estimated in the calibrated model, are still much higher than in the non-calibrated model. These modelled values are decisive for the final amount of nitrogen fixation. In summary, it seems that due to the very dry season, modelling of soil mineral nitrogen proved very difficult, which makes realistic estimations of nitrogen fixation by the grain legumes impossible.

P mobilization

The two location of the field trials in 2015 differ in the amount of available phosphorus in the soil at the beginning of the growing season. The experimental plot in Klazienaveen can be classified as neutral (Pw 52, P-PAE 5.4 and P-AL 34) but the Holten field is high in available phosphorus. At the end of the growing season, significant differences exist between Holten and Klazienaveen in P-AL and Pw, but not in P-PAE. No significant differences between available phosphorus are measured for the grain legumes, spring wheat and fallow (Table 2).

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Table 2. Soil P fractions after cultivation of grain legumes and wheat in 2015 field trials in Klazienaveen and Holten.

treatment		Klazienaveen			Holten		
		Pw	P-PAE	P-AL	Pw	P-PAE	P-AL
fallow		56,3	7,4	24,3	94,3	8,1	77,7
blue lupin	Iris	54,3	6,8	26,7	100,0	8,4	83,3
white lupin	Boros	48,7	5,4	29,7	93,7	7,6	80,7
faba bean	Imposa	44,0	4,9	25,7			
wheat		51,3	6,3	25,7	96,7	7,4	88,3
mean		**50,9**	**6,2**	**26,4**	**96,2**	**7,9**	**82,5**

At the beginning of the growing season in 2016, the availability of phosphate in the experimental field trial in Klazienaveen is low, but not very low. The Pw value of the soil is 35 (mg P_2O_5/l), the P-PAE 2,8 (mg P/kg), and the P-AL 28 (mg P_2O_5/100g). At the end of the growing season, the average Pw is 37.8 (mg P_2O_5/l), the P-PAE 3.2 (mg P/kg), and the P-AL 27.7 (mg P_2O_5/100 g). There are no significant differences between grain legumes, spring wheat, quinoa, buckwheat or fallow plots.

Table 3. Soil P fractions after cultivation of grain legumes, spring wheat, quinoa and buckwheat in 2016 field trials in Klazienaveen.

treatment	cultivar	Pw (mg P2O5/l)	P-PAE (mg P/kg)	P-AL (mg P2O5/100g)
spring wheat		39.3	3.3	29.0
blue lupin	Iris	38.3	3.0	29.0
quinoa	Atlas	38.3	3.4	26.7
fallow	braak	37.7	3.1	28.7
faba bean	Fuego	37.7	3.3	27.0
white lupin	Boros	37.7	2.9	29.7
buckwheat	Bw Cam	37.0	3.1	26.7
soybean	Viola	36.0	3.2	24.7
mean		**37.8**	**3.2**	**27.7**

Phosphorus mineral balance

In figure 3 the mineral balance for phosphorus is shown for white lupin, faba bean and spring wheat, as measured in the 2018 trials in Klazienaveen. Furthermore, the total phosphorus uptake of faba bean was measured the largest (13.3 kg P/ha), followed by spring wheat (12.9 kg P/ha) and white lupin (9.1 kg P/ha) (Table 7 in the appendix). Roots of

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spring wheat contained significantly more phosphorus than roots of lupin or faba bean. Total phosphorus in crop residues was measured largest in spring wheat as well.

Discussion

The data found in this trial contradict the hypothesis that crop residues of grain legumes like lupin and faba bean contribute more to phosphorus availability for subsequent cereal crops than a cereal crop itself. However, it should be noted that at the time of harvest, a considerable part of the roots of lupins and faba bean were already in the process of decomposition. Especially the lupin roots were decaying, as the plants had suffered from fungal diseases that had infected the roots. This might have contributed to overall lower phosphorus uptake by white lupin. It could be hypothesized that the decomposition of below-ground plant parts would be reflected in higher available phosphorus levels in soil. This, however, cannot be concluded from the experimental data. The immobilization of phosphorus by Fe and Al in the acid soil might happen so quickly that measurement of changes in plant-available phosphorus are not possible. This might be a reflection of the bias, also found in other experiments, where grain legumes are not always found to stimulate phosphorus uptake in the subsequent crop. This, in contrast to mixed cropping systems, where the effects of root exudates of grain legumes on nearby roots of cereals, seem to be more consequent in stimulating higher P uptake by cereal companion crops.

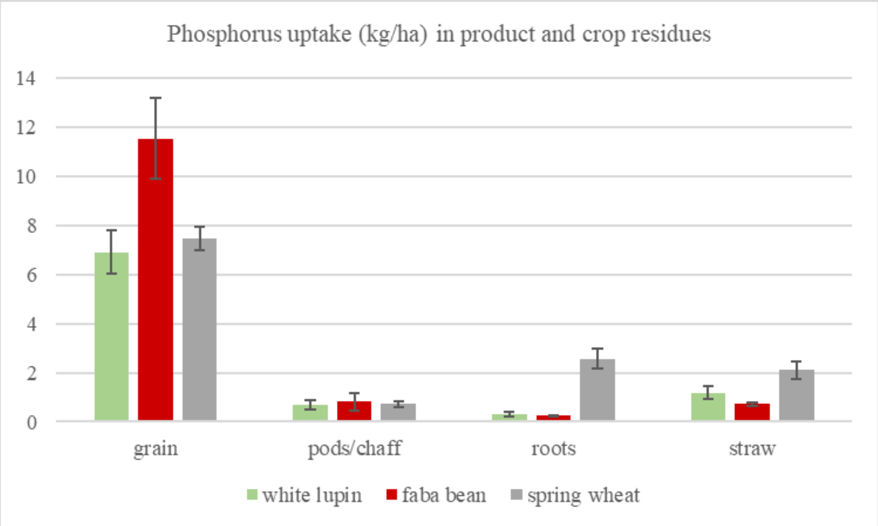

Figure 3. Phosphorus contents of harvested product and crop residues in kg/ha for white lupin, faba bean and spring wheat.

3.2 Effect of grain legumes on biodiversity

All pollinator inventories took place in the first half of June 2018. The Klazienaveen location is situated in a diverse environment, adjacent to a Natura-2000 peat natural reserve (Bargerveen). On the farm itself, natural landscape elements like windbreaks, dead wood (logs and tree trunks) and a wildflower strip can be found. The Bellingwolde location is

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surrounded by agricultural land, with a tree line on one side. A wildflower strip was sown along one of the field edges. The Oostwold location is also surrounded by agricultural land, but without any tree lines or wildflower strips.

A total of 43 species of pollinating insects (413 individuals) were found at the three locations. In Klazienaveen, bumblebees were the largest group of pollinators found in lupin flowers, followed by leaf cutter bees and honeybees. Hoverflies and bumblebees dominated among the pollinators of lupin in Bellingwolde. In Klazienaveen, honeybees, bumblebees and hoverflies were found in faba bean, while in Bellingwolde, hoverflies and bumblebees dominated. In Oostwold, honeybees dominated in faba bean, followed by bumblebees and hoverflies. One species of solitary mining bee (Andrena ovulata) was found in Oostwold, which, to our knowledge, has not been observed before in the province of Groningen. An overview of the species found in the three sites is given in Table 8 of the appendix.

The greatest diversity and the greatest number of pollinators was found in Klazienaveen, both in faba bean and lupin. In Oostwold, the diversity and number of individuals was found smaller in faba bean, followed by Bellingwolde. However, it should be noted that many of the faba beans at the Bellingwolde location were in a very late stage of flowering, which might have affected the lower number and diversity of pollinators found at the site. The presence or absence of groups of pollinators can be linked to the availability of landscape elements, which provide nesting and overwintering opportunities for wild bees and bumblebees. Where bumblebees may need old mouse holes to build their nest, leafcutter bees need beetle-riddled dead wood, earth, reed stalks or artificial bee hotels. The solitary mining bees on the other hand needs an open, sparely vegetated space on the ground to carve tunnels. Hibernation sites for queen bumblebees are probably not located in the same places were their colonies nested. Instead they use north facing slopes or areas under trees.

Although the list of observed pollinators in faba bean and lupin is limited to one year and three locations, together with the historical data on pollen analysis and flower visiting behavior of pollinators in the Netherlands, a preliminary overview was possible to conduct. This included wild pollinator species which may use faba bean and lupin as nectar and/or pollen source, and the measures which could be taken to enhance nesting opportunities (Table 4 and Table 5).

Some species, like the bivoltine Andrena flavipes and Andrena ovatula, produce more than one brood per year. Their flying period extends from March-September, instead of the univoltine species, which produce only one brood or generation per year. Furthermore, bumblebees need fouraging plants from early spring until autumn. Therefore, a continuous supply of flowering plants should be available throughout the year. It is also good to remember, that the flying distance of solitary bees is usually within a few hundred meters

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from their nesting sites. Bumblebees can fly distances up to 1800 m, but will prefer to stay within 500 m from their nesting sites, as the energy costs of large flight distances are high.

Table 4. Overview of potential pollinators of faba bean in the Netherlands and measures to increase nesting and overwintering opportunities.

Pollinator species	clifflet	bee bank	dead wood	nesting block
Andrena flavipes	x			
Andrena labiate	x	x		
Andrena ovulate	x	x		
Anthidium manicatum	x		x	x
Bombus hortorum				x
Bombus lapidaries				x
Bombus pascuorum				x
Bombus pratorum				x
Bombus terrestris				x
Halictus rubicundus	x	x		
Megachile centuncularis			x	x
Megachile ligniseca			x	
Megachile willughbiella	x		x	
Osmia bicornis			x	x
Osmia caerulescens	x		x	x
Stelis breviuscula			x	x

Table 5. Overview of potential pollinators of lupin in the Netherlands and measures to increase nesting and overwintering opportunities.

Pollinator species	clifflet	bee bank	dead wood	nesting block
Andrena ovatula	x	x		
Bombus hortorum				x
Bombus lapidaries				x
Bombus pratorum				x
Bombus terrestris				x

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Lasioglossum quadrinotatulum	x			
Megachile centuncularis			x	x
Megachile ligniseca			x	
Megachile willughbiella	x		x	
Osmia caerulescens	x		x	x

Historical data on pollen and pollinators of lupin and faba bean

Historical data on flower-visiting behavior of wild bee species were kindly provided by Menno Reemer (EIS-Netherlands) (Appendix, Table 9). With the exception of 4 observations, all data on lupin are gathered before 1950, and probably referring to bitter lupin species. Except for leaf cutter bees (Megachile), the list also mentions solitary mining species, which are rare or very rare in the Netherlands. Other rare species evident from the records were long-horned bees (Eucera longicornis), digger bees (Anthophora retusa), and the cavity nesting masonbee (Osmia uncinata). An interesting perspective would be to review whether lupin as a crop has the possibility to stimulate the abundance of these rare species.

Historical data on faba bean are even more scarce, as the majority of the data refers to the genus Vicia, which encompasses at least 12 different species in the Netherlands. Specific data on Vicia faba were only gathered in 2013 at one location in the Netherlands, with only reported species of honeybees and bumblebees.

A second historical data source applied in this study was provided by Jeroen Scheper (Alterra), which encompasses the pollen diet of wild bee species (Appendix, Table 10). The data indicates the percentage of Lupinus and Vicia pollen found in a total of 75 wild bee species. This data was also collected before 1950. Remarkable were the findings indicating that the diet of an individual of Andrena ovatula (the mining solitary bee for the first time recorded in Groningen), consisted of 21 % Vicia pollen. Similar findings were found of an individual Megachile circumcinta, carrying 34% of Lupin pollen. This historical data may indicate of the high potential of both lupin and faba bean flowers providing for pollinators. The results were not so evident in the present study due to the limited inventory, but it could provide interest for further research.

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4. Conclusion and next steps

In this study, the estimation of nitrogen fixation by faba bean and lupin has been done by the extended nitrogen difference method, and by soil nitrogen modelling with the NDICEA model. The extended difference method resulted in relatively low estimates for nitrogen fixation for both faba bean (122 kg N/ha) and lupin (80 kg N/ha), which may have been influenced by the extremely warm and dry climatic conditions, as well as the occurrence of root diseases in lupin. Modelling with NDICEA proved to be difficult. Though in the uncalibrated model, the estimation of nitrogen fixation corresponded the measurements received in the field (120 and 75 kg N/ha for faba bean and lupin), there was still a considerable gap between the measured and modelled soil mineral nitrogen. This gap is likely to be related to differences between the modelled versus measured soil moisture levels. Calibration of the model still resulted in an overestimation of soil mineral nitrogen, but also decreased the estimated amount of nitrogen fixation by the crops. This is due to the parameter in the model, which estimates the threshold level of soil mineral N below which all nitrogen is fixated. This was lowered from 15 kg N/ha in the uncalibrated model, to 6 kg N/ha in the calibrated model.

Total phosphorus uptake by grain legumes was estimated by calculating the sum of the phosphorus contents of the different plant parts. Total phosphorus contents of faba bean (13.3 kg P/ha) and lupin (9.1 kg P/ha) were not higher than those of spring wheat (12.9 kg P/ha). Roots of wheat contained more phosphorus than roots of faba bean or lupin. The low phosphorus contents might be a reflection of impeded root health (lupin) and dry weather conditions (faba bean), resulting in little overall root biomass of the grain legumes at the end of the growing season. Therefore, in this experiment, crop residues of grain legumes do not contribute more than cereal crop residues to phosphorus availability for the subsequent crop. The effect of root exudates or decomposition of crop residues on soil available phosphorus was not measurable. More insight of the effect of root exudates on phosphorus mobilization could be obtained through experiments on intercropping of grain legumes and cereals. Here the immediate effect of excess root exudates produced by legumes on the uptake of phosphorus by cereals might be measurable. Plant-available phosphorus was neutral to high on the soils tested, and differences might become more pronounced on phosphorus deficient or fixating soils.

The study on the abundance and diversity of pollinators of faba bean and lupin in PROTEIN2FOOD, showed the importance of both the presence of small landscape elements on farm level (windbreaks, tree trunks, logs, wildflower strips), and the connection with diverse natural areas, for the presence of a diverse community of pollinators. Further study should focus on the importance of wild pollinators on both yield level and yield stability in faba bean and lupin. The potential value of pollinators for these crops is important from both agricultural as well as broader biodiversity perspective. One of the

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added values of flowering protein crops on the landscape level, might be the potential of providing nectar and pollen sources for declining populations of wild pollinator species.

5. Delays and difficulties

The estimation of nitrogen fixation by the NDICEA model proved difficult due to extremely dry weather conditions in the 2018 experimental trial. The use of the extended difference method produced relatively low estimates of nitrogen fixation, but also here the weather conditions and occurrence of root diseases in white lupin is likely to have played a role. The phosphorus mobilization was measured in several years, but no differences in plant available phosphorus have been measured. Neutral to high phosphorus levels dominated the soils of the trial fields, which makes phosphorus mobilization in soil difficult to measure. Measurements of phosphorus mineral balances has been used as a second method, to overcome this difficulty. Also here, we found lower phosphorus amounts than expected, probably again related to dry weather conditions and diseases present in the lupin roots.

6. Impact and outreach

Although in its preliminary form, lupin will be included in the NDICEA model as a new grain legume. This makes further studies of the effects of grain legumes in cropping cycles on soil fertility, both on nitrogen use efficiency and on organic matter dynamics, possible. For farmers and advisors, this can be an important tool to assess and value the importance of grain legumes in their cropping cycles. Further calibration of the model with grain legumes is necessary, regarding the constraints experienced due to the extreme conditions in the 2018 season. Although the possible effects of phosphorus mobilization by legumes could not be shown with our study, there might be opportunities for further elaboration of this topic in the renewed interest in mixed cropping systems of legumes and cereals. The simultaneous cultivation of both legumes and cereals could provide more consistent results of the direct effect of legume root exudates on the phosphorus uptake of cereals, as has been shown in the literature. Especially on phosphorus deficient and fixating soils this combination of grain legumes and cereals could provide additional value.

The study performed on pollinator species in faba bean and white and blue lupin, indicates of a possible added value from larger-scale introduction of flowering grain legumes in the landscape, regarding to both the abundance and diversity of pollinators. The contribution of pollinators to both yield levels and yield stability in grain legumes should be subject of further research, and could stimulate farmers to support a diverse wild bee community. The role of specific legumes (faba bean, white and blue lupin) for specific endangered species of pollinators could also be addressed in future research.

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7. Literature

Cheng, L., B. Bucciarelli, J. Liu, K. Zinn, S. Miller, J. Patton-Vogt, D. Allan, J. Shen, and C.P. Vance (2011) White lupin cluster root acclimation to phosphorus deficiency and root hair development involve unique glycerophosphodiester phosphodiesterases. Plant Physiology 156: 1131–114

Ehlert, P.A.I., S.L.G.E. Burgers, D.W. Bussink, E.J.M. Temminghoff, P.J. van Erp and W.H. van Riemsdijk. (2007) Deskstudie naar de mogelijkheden van het aanwijzen van fosfaatarme gronden op basis van P-PAE. Stand van zaken 2006. Wageningen, Alterra, Alterrra-rapport 1458, 80p.

Espinosa, D. (2014) Role of legume break crops in mobilising soil phosphorus (P) for wheat. 2014 Victorian GRDC Grain Research Update for Advisers: 121-123

Hauser, S. (1990) Estimation of symbiotically fixed nitrogen using extended N difference methods. In: Biological nitrogen fixation and sustainability of tropical agriculture. AABNF Proceedings, Nigeria

Horst, W., Kamh, M., Jibrin, J. and V.O. Chube (2001) Agronomic measures for increasing P availability to crops. Plant and Soil 237: 211-223

Li, L.S-M. Li, J-H. Sun, L-L. Zhou, X-G. Bao, H-G. Zhang, and F-S. Zhang (2007) Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. PNAS 104(27): 11192–11196

Makking, G.F. (1957) Testing the Penman formula by means of lysimeters. Journal of International Water Engineering 11, 277-288

Nayak, G.K., S.P.M. Roberts, M. Garratt, T.D. Breeze, T. Tscheulin, J. Harrison-Cripps, I.N. Vogiatzakis, M.T. Stirpe and S.G. Potts. (2015) Interactive effect of floral abundance and semi-natural habitats on pollinators in field beans (Vicia faba). Agriculture, Ecosystems and Environment 199:58-66

Neugschwandtner,R., K. Ziegler, S. Kriegner, H. Wagentristl and H-P Kaul (2015) Nitrogen yield and nitrogen fixation of winter faba beans, Acta Agriculturae Scandinavica, Section B, Soil & Plant Science: 9p

Nuruzzaman, M., H. Lambers, M.D.A. Bolland and E.J. Veneklaas (2005) Phosphorus uptake by grain legumes and subsequently grown wheat at different levels of residual phosphorus fertilizer. Australian Journal of Agricultural Research, 56: 1041–1047

Peoples, M.B., J. Brockwell, D.F. Herridge, I.J. Rochester, B.J.R. Alves, S. Urquiaga, R.M. Boddey, F.D. Dakora, S. Bhattarai, S.L. Maskey, C. Sampet, B. Rerkasem, D.F. Khan, H. Hauggaard-Nielsen and E.S. Jensen (2009) The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis 48:1-17

Rose, T.J., B. Hardiputra and Z. Rengel (2010) Wheat, canola and grain legume access to soil phosphorus fractions differs in soils with contrasting phosphorus dynamics. Plant Soil,, 326: 159-170

Van der Burgt, G.J.H.M., G.J.M. Oomen, A.S.J. Habets and W.A.H. Rossing (2006) The NDICEA model, a tool to improve nitrogen use efficiency in cropping systems. Nutrient Cycling in Agroecosystems 74: 275 –294.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635727.

8. Appendix

8.1 Additional tables

Table 6. Mean nutrient contents in harvested grain and crop residues for white lupin Boros, faba bean Fuego, and spring wheat Lavett. Values in g/kg DM, except for Mn, B, Cu, Zn and Fe in mg/kg DM, and DM in g/kg fresh product.

	white lupin				**faba bean**				**spring wheat**			
	grain	**pods**	**roots**	**straw**	**grain**	**pods**	**roots**	**straw**	**grain**	**chaff**	**roots**	**straw**
DM	892	822	704	689	882	848	568	742	875	885	685	827
N	52,7	13,7	8,8	12,9	46,5	19,0	8,0	8,0	17,6	9,8	6,1	3,2
P	3,4	0,6	0,3	0,6	3,3	1,0	0,3	0,4	3,5	1,4	0,6	1,2
K	12,0	19,4	14,3	25,1	12,2	17,5	9,3	28,2	4,9	9,2	7,0	17,0
Ca	2,4	6,3	3,1	4,3	1,1	10,5	2,9	5,8	0,3	4,8	2,4	1,8
Mg	1,8	2,2	0,9	1,4	1,3	3,3	0,7	3,0	1,2	2,0	0,7	1,1
S	2,2	0,9	0,6	1,3	1,9	1,3	0,5	0,7	1,4	1,5	0,8	1,3
B	16,4	23,4	11,1	18,9	11,7	19,4	11,3	22,9	2,2	10,9	2,8	7,8
Cu	3,4	2,3	2,1	1,9	4,7	4,1	2,0	1,3	3,8	3,8	2,0	1,9
Fe	29	430	212	60	43	991	140	51	32	1081	876	120
Mn	133	215	99	156	21	90	16	42	58	270	89	126
Zn	41	26	32	34	51	51	20	26	38	40	15	25

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Table 7. Mean total nutrient uptake as measured in harvested product and crop residues. Values in kg/ha, except for Mn, B, Cu, Zn and Fe (g/ha), and DM (tonnes/ha).

	white lupin					**faba bean**					**spring wheat**				
	grain	pods	roots	straw	**total**	grain	pods	roots	straw	**total**	grain	chaff	roots	straw	**total**
DM	2.1	1.1	0.9	1.8	**5.9**	3.5	0.8	0.9	1.7	**6.8**	2.2	0.5	4.3	1.8	**8.7**
N	108	15	8	24	**154**	161	15	7	13	**197**	38	5	26	6	**75**
P	6.9	0.7	0.3	1.2	**9.1**	11.5	0.8	0.2	0.7	**13.3**	7.5	0.7	2.6	2.1	**12.9**
K	25	20	13	47	**104**	42	13	9	47	**111**	11	5	30	31	**76**
Ca	5.0	6.9	2.8	8.0	**22.7**	3.9	8.0	2.7	9.5	**24.0**	0.6	2.4	10.2	3.3	**16.5**
Mg	3.6	2.4	0.8	2.7	**9.5**	4.5	2.5	0.6	4.9	**12.5**	2.6	1.0	2.9	2.1	**8.6**
S	4.4	1.0	0.5	2.5	**8.4**	6.5	1.1	0.4	1.1	**9.1**	3.0	0.7	3.4	2.4	**9.6**
B	34	25	10	35	**103**	41	15	10	38	**104**	5	5	12	14	**36**
Cu	7.1	2.5	2.0	3.4	**15.0**	16.2	3.4	1.8	2.1	**23.5**	8.2	1.9	8.5	3.4	**21.9**
Fe	59	469	196	108	**832**	149	754	130	84	**1117**	69	534	3715	219	**4537**
Mn	282	242	91	294	**909**	74	76	15	70	**235**	123	138	381	231	**874**
Zn	84	29	29	63	**205**	176	43	18	43	**280**	82	20	63	45	**211**

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Table 8. Number of pollinating species found at two observation days (along six 25-meter transects) at the locations Klazienaveen, Bellingwolde and Oostwold.

	Klazienaveen		Bellingwolde		Oostwold
	lupin	**faba bean**	**lupin**	**faba bean**	**faba bean**
Megachile willughbiella	10	1			
Megachile centuncularis	1				
Andrena ovatula					1
Apis mellifera	10	38	1	1	29
Bombus terrestris[1]	22	23	7	4	5
Bombus pratorum	1	2			
Bombus pascuorum	9	5	3	5	11
Bombus lapidarius	1		4		3
Episyrphus balteatus	6	19			3
Syrphus ribesii	3	3	10	1	5
Sphaerophoria scripta	11	5	26	10	8
Syritta pipiens		3			
Eupeodes corollae	4	1			1
Melanostoma mellinum	3				
Paragus haemorrhous	1				
Eristalis arbustorum	1		1		2
Eristalis intricaria	1	1			
Eristalinus sepulchralis		1			
Helophilus trivittatus	3	1			
Helophilus pendulus			2		
Pieris rapae	3	1			8
Pieris brassicae					1
Pieris napi	1	1			
Plebejus argus	1				
Gonepteryx rhamni		1			
Maniola jurtina		1			
Autographa gamma	1	2	4		
Coccinella septempunctata		3	4	1	1
Harmonia axyridis		4			5

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	Klazienaveen		Bellingwolde		Oostwold
	lupin	**faba bean**	**lupin**	**faba bean**	**faba bean**
Propylea quatuordecimpunctata			1	1	
Phyllopertha horticola		1			
Anomala dubia		2			
Notoxus monoceros			3		
Sicus ferrugineus	1				
Physocephala rufipes	1				
Chloromyia formosa					1
Chrysopidae indet.		1	6		
Ichneumonidae indet.		3	1		3
Eumenes coarctatus		1			
Cerceris arenaria		3			
Philanthus triangulum		1			
Ancistrocerus gazella	1				
Ectemnius spec.			1		
Polistes dominula	1	1			

[1] Species belonging to the Bombus terrestris/magnus/lucorum/cryptarum group

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Table 9. Historical records of flower-visiting behavior of wild bees on Vicia faba and Lupinus in The Netherlands (data provided by Menno Reemer, EIS Netherlands).

plant species	wild bee species	location	year	month	day	sex	number
Lupinus	Andrena ovatula	Babberich	1945	05		female	1
Lupinus	Anthophora retusa	Wahlwiller	1985	05	29	male	1
Lupinus	Eucera longicornis	Babberich	1940	05	21	male	1
Lupinus	Megachile alpicola	Mariaveen	1947	05	30	male	1
Lupinus	Megachile centuncularis	Aerdenhout	1931	07		female	4
Lupinus	Megachile centuncularis	Tilburg	1991	07	26	female	1
Lupinus	Megachile circumcincta	Aerdenhout	1926	06	20	female	2
Lupinus	Megachile circumcincta	Baarn	1941	06	24	male	7
Lupinus	Megachile circumcincta	Aerdenhout	1926	06	20	female	1
Lupinus	Megachile circumcincta	Bilthoven	1919	06	08	female	2
Lupinus	Megachile circumcincta	Bilthoven	1919	06	10	female	1
Lupinus	Megachile circumcincta	Helenaveen	1946	05	25	female	2
Lupinus	Megachile circumcincta	Ulvenhout	1944	05	28	male	1
Lupinus	Megachile willughbiella	Baarn	1939	06	05	male	1
Lupinus	Megachile willughbiella	Deurne	1946	05	25	male	1
Lupinus polyphyllus	Eucera longicornis	Beyart	1953	07	20	female	1
Lupinus polyphyllus	Eucera longicornis	Wolfhaag	1975	06	07	male	2
Lupinus polyphyllus	Megachile ligniseca	Babberich	1940	06	20	male	2
Lupinus polyphyllus	Osmia uncinata	Baarn	1942	06	03	female	1
Vicia	Andrena lathyri	Westervoort	1992	05	23	female	2
Vicia	Andrena lathyri	Westervoort	1992	05	23	female	2
Vicia	Anthophora retusa	Maastricht	1989	05	15	female	1
Vicia	Bombus hortorum	Zaltbommel	2001	06	29	female	1
Vicia	Bombus lapidarius	Zaltbommel	2001	06	29	female	1
Vicia	Bombus pascuorum	Leeuwarden	2013	06	05	nd	1
Vicia	Bombus pascuorum	Den Haag	2016	05	11	female	1
Vicia	Bombus pascuorum	Den Haag	2016	05	12	female	1
Vicia	Chalicodoma ericetorum	Baarn	1943	05	29	male	1
Vicia	Chalicodoma ericetorum	Itteren	2015	06	07	male	1
Vicia	Colletes halophilus	Paal	1980	09	21	female	1

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plant species	wild bee species	location	year	month	day	sex	number
Vicia	Eucera longicornis	Babberich	1942	05	27	female	1
Vicia	Eucera longicornis	Kannerbos	1984	06	11	female	1
Vicia	Eucera longicornis	Kannerbos	1989	05	17	male	1
Vicia	Eucera longicornis	Kannerbos	1989	07	11	female	2
Vicia	Eucera longicornis	Kannerbos	1990	06	29	female	2
Vicia	Eucera nigrescens	Kannerbos	1986	06	20	female	2
Vicia	Eucera nigrescens	Kannerbos	1989	05	14	female	1
Vicia	Eucera nigrescens	Heerlen	1929	06	19	male	1
Vicia	Eucera nigrescens	Epen	1947	05	11	male	1
Vicia	Halictus confusus	Schrievhei	1984	07	09	female	1
Vicia	Megachile willughbiella	Wolder	1990	06	24	female	1
Vicia	Megachile willughbiella	Simpelveld	1990	06	01	male	1
Vicia	Megachile willughbiella	Beyart	1998	06	10	female	1
Vicia	Megachile willughbiella	Itteren	2015	06	07	male	3
Vicia	Osmia leaiana	Itteren	2015	06	07	male	1
Vicia	Stelis breviuscula	Helenaveen	1947	06	20	female	1
Vicia faba	Apis mellifera	Leeuwarden	2013	06	26	nd	1
Vicia faba	Apis mellifera	Leeuwarden	2013	06	26	nd	1
Vicia faba	Bombus pascuorum	Leeuwarden	2013	07	18	nd	1
Vicia faba	Bombus terrestris	Leeuwarden	2013	06	26	nd	1
Vicia faba	Bombus terrestris	Leeuwarden	2013	06	26	nd	1
Vicia faba	Bombus terrestris	Leeuwarden	2013	06	26	nd	1
Vicia faba	Bombus terrestris	Leeuwarden	2013	06	26	nd	1

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Table 10. Historical data on percentage of Vicia and Lupinus pollen in the diet of wild bee species in The Netherlands (data provided by Jeroen Scheper, Alterra)

Wild bee species	% pollen Lupinus	% pollen Vicia
Andrena flavipes		4.2
Andrena labiata		6.3
Andrena ovatula		21.1
Anthidium manicatum		9.7
Anthophora retusa		5.2
Bombus hortorum	4.1	1.8
Bombus humilis		5.7
Bombus lapidarius	4.4	0.3
Bombus pascuorum		2.1
Bombus pratorum	4.4	2.2
Bombus ruderatus	9.0	2.7
Bombus terrestris	7.0	
Halictus confusus		1.1
Halictus rubicundus		0.3
Hoplitis claviventris		6.1
Lasioglossum punctatissimum		5.0
Lasioglossum quadrinotatulum	7.7	
Megachile centuncularis		5.6
Megachile circumcincta	34.1	1.5
Megachile leachella		17.1
Megachile ligniseca		1.0
Megachile maritima	10.5	8.0
Megachile willughbiella	8.5	5.0
Osmia bicornis		2.6
Osmia caerulescens	4.5	12.2

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8.2 Description of soil phosphorus fractions and classification of Dutch soils

Three methods have been used to measure the P-fractions in soil. P-PAE and Pw are both methods estimating the plant-available amount of phosphorus in the soil, while the P-AL method estimates the long-term plant availability.

The P-PAE (Phosphorus-Plant Available Elements) has been developed by Eurofins since 2003, and is derived from the 0.01 $CaCl_2$ method. It is a multi-element extraction method, where in one extract, all nutrients and the pH are determined.

The Pw (Phosphorus-water) is an extraction method, which has been developed in 1968, as an alternative for the P-AL (Phosphorus – Ammonium Lactate) method. The P-AL method, did not provide sufficient relation with the actual plant-available phosphorus, especially for arable crops, and sand- and former peat soils. The Pw is based on a 1:60 volume extraction of (dried) soil in water. Before the actual extraction takes place, re-wetting of the dried soil sample is done.

The correlation between Pw and P-PAE has been investigated, but as both methods are prone to measurement errors, the relationship between Pw and P-PAE is ambiguous. A small percentage of Dutch soils would be classified as phosphate-poor/fixating according to the P-PAE method, while not being classified as such with the Pw method, and vice versa (Ehlert et al, 2006).

The P-AL method has no direct relationship with plant-available phosphorus, especially in arable crops, but it is used as a measure for phosphorus availability in grassland. The P-AL method uses a moderately acid extraction with ammoniumlactate (AL) and acetic acid (pH 3.75). It can be used as a measure for the longer-term available plant phosphorus. Phosphorus availability based on Pw (arable land) and P-AL (grassland) are used to classify Dutch soils (Table 11).

Table 11. Classification of phosphorus status of Dutch soils based on Pw or P-AL.

grassland		**arable land**	
P-AL (mg P2O5/100 g soil)	**P classification**	**Pw (mg P2O5/l soil)**	**P classification**
< 16	poor or fixating	< 25	poor or fixating
< 27	low	< 36	low
27-50	neutral	36-55	neutral
> 50	high	> 55	high

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