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RESEARCH ARTICLE

Oat, corncockle, and lupine growth affects resin-extractable soil phosphorus and soil microbial properties differently[#]

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Abstract

Background: Improved use of legacy phosphorus (P) in agricultural soils is requested to reduce the need for P fertilizers. Adapted use of cover crops (CCs) may be a promising tool to support this.

Aim: We estimated the P allocation to roots and shoots of oat (*Avena sativa*, cv Posedion), corncockle (*Agrostemma githago*), and lupine (*Lupinus angustifolius*, cv Iris) and their effect on soil enzyme activity, microbial community structure, and indices of plant-available soil P.

Methods: We grew the CCs in pots on soils with low- and medium-P status. After 40 days, we measured P, N, and C uptake in shoots and roots; soil microbial C, N, and P; and pH and inorganic P extracted with water (P_{H2O}) and anion-exchange resins (P_{resin}). Soil microbial activity and community structure were assessed by determining phosphomono- and phosphodiesterase, β-glucosidase, and N-acetyl-glucosaminidase activity and by extraction of phospholipid and neutral lipid fatty acids (PLFAs and NFLAs).

Results: Corncockle and lupine took up similar amounts of P, but corncockle had an almost fourfold higher concentration of P. In the low-P soil, the activity of phosphomonoesterase and soil microbial biomass (total microbial PLFA) were higher after lupine. CCs did not affect P_{H2O}, but after corncockle, P_{resin} was reduced in the medium-P soil. Oat enhanced the presence of arbuscular mycorrhizal fungi in soil.

Conclusions: Our results thus suggest that CC species with different P uptake and P uptake strategies can modify aspects in soil of potential importance for the P supply of the following main crop.

KEYWORDS
cover crops, green manure, mycorrhiza, nutrient cycling, PLFA

1 | INTRODUCTION

Phosphorus (P) is an essential plant macronutrient, which is fairly immobile in most soils. This necessitates applications of P fertilizers to meet crop requirements and this P originates primarily from nonrenewable, mined rock phosphates (Elser & Bennett, 2011). In areas with long-term intensive agriculture, applications of P in surplus to what is being removed by the crops have caused P to accumulate (Rubæk et al., 2013). This legacy P poses a risk for the aquatic environment in areas where excess precipitation passes over or infiltrates through P-enriched soil layers before it reaches surface waters (Schoumans et al., 2014). It is, however, also a valuable resource for future crop

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production in regions where the application rate of P to soil is being subject tighter and tighter regulations as it is the case in several European countries (Amery & Schoumans, 2014). Improved use of soil legacy P has particular relevance for organic farmers, who face additional limitations regarding which nutrient sources they can apply (Moller et al., 2018). It is therefore essential to look for management options that improve crop uptake of soil P in all cropping systems and on soil with different soil P status (Frossard et al., 2000).

A cover crop (CC) is a crop grown between two main crops, its biomass remaining in the field. They can serve multiple purposes such as weed suppression, erosion control, retention of nutrients, and increased soil fertility (Dabney et al., 2001; Snapp et al., 2005; Sturm et al., 2018). Thus, CCs could present an intermediate storage of P for the next crop, but in climates where the CCs are exposed to repeated freezing and thawing during winter this may increase losses of dissolved P from catch crops (Aronsson et al., 2016). In Denmark, CCs serve as a mandatory measure for farmers to reduce nitrate leaching and retain nitrogen in the root zone for utilization by the following crop. Approved species for this purpose include chicory, crucifers, and grasses (including cereals) (Aronsson et al., 2016). The use of CCs in the cropping system can potentially alter soil P dynamics for the benefit of the main crop (Arrobas et al., 2015; Hallama et al., 2019; Mbuthia et al., 2015; Soltangheisi et al., 2020). Yet, there has been little focus on how a CC implemented to reduce nitrate leaching affects P turnover and indices of plant-available P in the soil. This issue is becoming increasingly relevant for organic as well as conventional farmers because of new regulations in Denmark on how much P can be applied to land (Sørensen et al., 2019) to encourage farmers to make better use of soil P.

CC species differ in their strategies to acquire P. These strategies include the formation of mycorrhiza, modification of the root system, and release of root exudates, such as organic acids (Hinsinger, 2001; Wendling et al., 2016; White et al., 2013). Furthermore, plant species have different P requirements and biomass productions, which may lead to differences in P uptake, tissue concentrations, and allocation within the plant (Asher & Loneragan, 1967).

CCs may improve the use of soil P more efficiently in low-P systems, but the CC effects on P in soils with different soil P status are generally understudied (Hallama et al., 2019), especially studies on soils of similar origin and cropping history, where soil P status is the only factor varying. There is therefore a need to investigate CC effects on such soils. Furthermore, the soil P status will likely affect the plants root:shoot ratio, where P deficiency has been found to increase this ratio, for example, in Atkinson (1973) and Mollier and Pellerin (1999).

Microorganisms play an important role in the turnover of P in the soil (Bünemann et al., 2012; Richardson & Simpson, 2011; Stewart & Tiessen, 1987), and in agricultural systems the abundance, structure, and activity of the microbial community are sensitive to soil properties and management factors such as crop rotation and management of CCs (Cloutier et al., 2020). The use of a CC can increase microbial enzymatic activity in the soil (Brennan & Acosta-Martinez, 2019; Rankoth et al., 2019) and hence the turnover of nutrients. Measuring enzyme activities provides information on the capacity of the soils to mineralize plant residues, and the activity of enzymes is known to quickly respond to changes in soil management and crop growth (Karaca et al., 2010; Nannipieri et al., 2011). For example, C cycling enzyme responds within weeks to months to the additional of litter material or the change of tillage treatments (Kandeler & Böhm, 1996; Poll et al., 2003). Growing CCs can furthermore affect the microbial community structure (Fernandez et al., 2016; Nevins et al., 2018; Rankoth et al., 2019). However, studies on how different CC species affect the microbial community and activity in soils with different soil P status are lacking.

The aim of this study was to investigate how different CC species with contrasting P requirements and acquisition strategies grown at low and medium soil P status affect the microbial community structure and activity and indices of plant-available P in soil. We selected oat (*Avena sativa*) because it is a mycorrhiza-forming cereal already approved and used as a nitrate-catching CC in Denmark. Corncockle (*Agrostemma githago*) was selected because screening trials in Denmark have revealed a remarkably high P concentration in the aboveground plant tissue and it has performed well as a nitrate-catching CC (Christensen, 2018). Lupine (*Lupinus angustifolius*) is a legume capable of fixing N and therefore desired as a CC by organic farmers, but it is also known to access sparingly soluble P forms (Lambers et al., 2013). We used two soils, one with low and the other one with a medium soil P status.

We hypothesized that:

1. Shoot biomass production and P uptake will be larger in medium-P soil than low-P soil for all CC species, and a low-P soil will result in larger allocation of biomass and P to roots than in medium-P soil;
2. The CC species modify microbial community structure and function of the microorganisms involved in the turnover of P in soil differently and the effects of the CC species will be more pronounced in the low-P soil than in the medium-P soil;
3. The species-specific differences in growth and nutrient uptake and effects on soil microorganisms lead to changes in the two indices of plant-available soil P—water- and resin-extractable P (P_{H2O} and P_{resin}).

2 | MATERIALS AND METHODS

2.1 | Soil preparation

The soil for the pot experiment was collected on March 20, 2018 from the plough layer in a long-term experiment on lime and P application located in Jyndevad (54°54′ N, 9°07′ E), Denmark. The soil is a coarse sandy soil (4.2% clay, 4.1% silt, 16.7% fine sand, and 75% coarse sand) according to Nielsen and Møberg (1985) and was classified by Møberg and Nielsen (1986) as an Orthic Haplohumod according to U.S. Soil taxonomy (Soil Survey Staff, 1975). The field has been cropped with barley for several decades. We selected soil from two treatments that had received the same amount of lime (8 Mg ha^{-1} applied with 6–9 years intervals) with either no application of P (low-P soil) since 1942 or with a yearly application of 15.6 kg P ha^{-1} (medium-P soil) as triple

superphosphate. Lime was last applied in 2013. Olsen-P values were 11 and 21 mg P kg^{-1}, respectively, corresponding to low and medium soil P status according to the recommendation scheme for P fertilization on this soil type in Denmark (Jordan-Meille et al., 2012). Total P in the upper 20 cm was 330 and 420 mg kg^{-1} for the low- and medium-P soil, respectively. Oxalate-extractable P was 260 and 360 mg kg^{-1} for the low- and medium-P soil, respectively (Azeez et al., 2020). Details on the long-term experiment can be found in Rubæk (2008) and in Azeez et al. (2020). Field-moist soil was thoroughly mixed and sieved through a 1 × 1 cm screen. Moist soil was packed in pots made of PVC tubes with an inner diameter of 11 cm and height of 40 cm. The pots were packed to a bulk density of 1.3 g cm^{-3} of dry soil (3824 g dry soil per pot), based on a dry matter determination of a subsample dried at 105°C.

2.2 | Experimental setup

Three CC species were sown on May 3, 2018. All CCs and a no-crop (bare soil) treatment were established in both low- and medium-P soil, and all combinations were replicated four times (total of 32 pots). Lupine seeds were inoculated with *Bradyrhizobium* spp. (Lupinus). The pots were randomly placed in four blocks on racks in an outdoor facility for pot experiments at Aarhus University, Foulum. Seven seeds of each CC were sown, and 6 days after sowing (DAS) the number of plants were reduced to four for oat and corncockle, and three for lupine. The pots were irrigated with demineralized water to 60% of the water-holding capacity (WHC; determined on packed soil in pots) every 1–3 days. The pots were exposed to ambient weather conditions including mild rain, but during heavy rainstorms a roof covered the pots temporarily. Twenty-one DAS irrigation was increased to 70% of WHC in order to maintain sufficient water supply. The mean temperature of the growth period was 17°C, and the pots were exposed to six rainfall events with 2.8-, 4.2-, 1.7-, 1.2-, 2.9-, and 9.6-mm precipitation. Macro- and micronutrients were applied as nutrient solution on 11 or 13 DAS at the following rates (g m^{-2}): 3.0 N (as NH_4NO_3), 12.5 K (as K_2SO_4), 10.0 Ca (as $CaCl_2$), 5.0 Mg (as $MgCl_2$), 0.245 Mn (as $MnSO_4$), 0.196 Zn (as $ZnSO_4$), 0.054 B (as H_3BO_3), 0.196 Cu (as $CuSO_4$), 0.005 Co (as $CoCl_2$), and 0.025 Mo (as $NaMoO_4$).

Growth was terminated 40 DAS by cutting the shoots at the soil surface. The soil from each pot was retrieved by sieving through a 4-mm sieve. The sieved soil was thoroughly mixed and representative soil samples were taken for storage at 2°C for analyses of chemical properties and at −18°C for analysis of microbial properties. Roots were gently washed free from soil and stored at 2°C.

2.3 | Soil pH and nutrients in soil and microbial biomass

Soil pH was measured on approximately 5 g moist soil in a 0.01 M $CaCl_2$ solution using a pH electrode. Mineral Nitrogen (N_{min}) was determined by mixing 10 g moist soil into 40 mL 1 M KCL solution for 30 min. The concentration of nitrate and ammonium was determined in the filtered extracts by flow colorimetry using an Autoanalyzer III (Bran + Luebbe GmbH, Nodersted, Germany).

Water-extractable P (P_{H2O}) was determined on 5 g moist, sieved (<2 mm) soil shaken for 17 h at 20 rpm with 50 mL demineralized water. Soil and water were separated by centrifugation for 10 min at 1831 × *g*. P_{resin} and microbial biomass P were determined on sieved (<2 mm), moist soil (corresponding to 2 g dry soil dried at 105°C) by extraction with anion-exchange resin membranes in 30 mL demineralized water either without (P_{resin}) or with addition of 1 mL hexanol for lysis of microbial cells as described by Kouno et al. (1995). The solution was shaken for 17 h at 20 rpm on an end-over-end shaker. P_{resin} in soil equaled the extracted amount of P in the samples without hexanol addition, and microbial P (P_{mic}) was calculated as the difference in P extracted from parallel samples with and without hexanol addition. The P_{mic} was calculated assuming an identical P recovery factor of 0.82 for both soils. This factor was derived from preliminary studies on soil from the same site and with varying soil P status. The P content in the P_{H2O} extract and the eluate of the resins retrieved after extractions were determined colorimetrically using the molybdate blue technique as described in the ISO protocol for analyzing inorganic phosphate in water samples (ISO, 2004).

Microbial C (C_{mic}) and N (N_{mic}) were determined by fumigation of 10 g moist soil in chloroform for approximately 24 h. Fumigated and unfumigated samples were extracted with 40 mL 0.5 K_2SO_4 solution, shaken for 30 min and centrifuged at 4231 × *g* for 30 min. The C content in the extract was measured on a TOC analyzer (ASI-V, Shimadzu, Duisburg, Germany) and the N content was measured on an autoanalyzer (AA500, SEAL Analytical, Germany) following the principle of Houba et al. (1987). Microbial biomass C was calculated as the difference in C concentration between the fumigated and nonfumigated samples multiplied by the extraction volume, and then divided by the soil dry matter content and a conversion factor of 0.45 as described by Joergensen (1996). Microbial biomass N was calculated the same way, but using a conversion factor of 0.54 (Joergensen, 1996). The extracted amount of C from the unfumigated sample was additionally used as a measure of labile C in soil. For all soil analyses where moist soil was used, results were converted into the dry matter equivalent of the specific soil sample.

2.4 | Plant analyses

Plant material (roots and shoots) was dried at 60°C until no further loss of weight (3 days) for dry matter yield determination. Dried samples were ground into a fine powder using a ball mill. For determination of P concentration, the plant powder was digested with 3 mL H_2O_2 (9.7 M) and 6 mL HNO_3 (14.3 M) in a microwave and analyzed by using ICP-OES (Inductively Coupled Plasma–Optical Emission Spectroscopy) according to Liu et al. (2013). The concentrations of C and N in the plant material were determined by weighing 250 g of ground material on a Vario Max Cube dry combustion system (Elementar Analysensysteme GmbH, Germany). There was not enough material of each sample to quantify soil remnants on root samples.

2.5 | Microbial community structure

The structure of the microbial community was determined by extraction of phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) biomarkers, assigning them to specific groups of microorganisms. PLFAs and NLFAs were extracted from 4 g of frozen moist soil using the reagent of Bligh and Dyer (1959) (citrate buffer:chloroform:methanol in the volume ratio of 1:2:0.8) for 2 h. Citrate buffer and chloroform were added for phase separation, and after separation the organic phase was evaporated under N_2 flow. The lipids were fractionated in silica columns. NLFAs were extracted by flushing with chloroform, and PLFAs by a flush of methanol. Methanolysis of PLFAs and NLFAs was performed by the addition of a methanol–toluol solution and 0.2 M methanolic KOH. After incubation for 15 min at 37°C, a hexane-chloroform solution was added, followed by the addition of acetic acid and deionized water. Fatty acids were measured by gas chromatography and identified by comparison to a mixture of known fatty acids. PLFA biomarkers were for gram-positive bacteria i15:0, a15:0, i16:0, and i17:0, for gram-negative bacteria cy17:0 and cy19:0, and for fungal biomass 18:2ω6,9. Total microbial PLFA was determined by the sum of the biomarkers for gram-positive bacteria, gram-negative bacteria, fungi, as well as 16:1ω5, 15:0, and 16:1ω7 PLFA. The NLFA biomarker 16:1ω5 was used to estimate the abundance of arbuscular mycorrhizal fungi (AMF) (Frostegård & Bååth, 1996).

2.6 | Enzyme activity

The activities of the enzymes phosphomonoesterase, phosphodiesterase, β-glucosidase, and N-acetyl-glucosaminidase were determined using a fluorometric substrate assay as described by Marx et al. (2001) and modified by Poll et al. (2006). The following substrates were used: 4-methylumbelliferyl-β-D-glucoside for ß-glucosidase, 4-methylumbelliferyl-N-acetyl-βD-glucosaminidase for N-acetylglucosaminidase, 4-methylumbelliferyl-phosphate for acid phosphomonoesterase (hereafter phosphatase), and bis-(4-methylumbelliferyl)-phosphate for phosphodiesterase activity. One gram of frozen moist soil was dispersed by ultrasonication (50 J s^{-1}, UP200S, Hielscher GmbH, Germany) for 2 min in 40 mL of demineralized water. The soil solution (50 µL) was added to wells of a microplate together with 100 µL of a solution of fluorogenic substrate and 50 µL of 0.1 M MES buffer [2-(N-morpholino)ethanesulfonic acid, pH 6.1]. For each enzyme and soil, standards were prepared to create specific standard curves. The standards consisted of 50 µL soil solution with combinations of buffer and increasing concentrations of fluorogenic substrate. All microplates were placed in an incubator at 30°C, and the fluorescence was measured on a microplate reader (FLX 800, Microplate Fluorescence Reader, Bio-Tek Instruments Inc., VT, USA) at 360/460 nm after 0, 30, 60, 120, and 180 min of incubation. For each soil sample, all enzyme analyses were carried out in triplicate. Enzyme activity was determined by relating the linear slope of emitted fluorescence to the standards (Poll et al., 2006).

2.7 | Calculations and statistical analysis

The uptake of P was calculated for shoot and root by multiplying the dry matter production in each plant part with the P concentration. As a measure of the quality of the plant tissue, the nutrient ratios were calculated based on weight.

Data analysis was conducted using R version 3.5.1 (R Core Team, 2019). The measured parameters were analyzed using linear models with two explanatory variables: soil P status (medium and low) and CC species (oat, corncockle, and lupine). Models were checked for homogeneity of variance and normal distribution using visual interpretation of residuals plots and QQ-plots. Due to right-skewness of the AMF abundance (NLFA biomarker 16:1ω5) and the activity of phosphomonoesterase, these variables were analyzed using generalized linear models with the identity link function and gamma distribution. Two-way ANOVA was used to evaluate the significance of the main and interaction effects. Pairwise comparisons were conducted using Tukey's test and the CLD function in the R package *emmeans* (Lenth, 2019). Significance was declared when $p < 0.05$. All figures and tables display estimated means from the models, 95% confidence intervals, and letters showing significant differences among treatments.

3 | RESULTS

3.1 | Dry matter production, P uptake, and C:N and C:P ratios in shoots and roots

Lupine produced more than double the amount of shoot dry matter compared to oat and corncockle. Nodules were observed on the roots of lupines. Growth of CC in medium-P soil resulted in a significantly higher production of dry matter compared to low-P soil (Tables 1 and 2). Root dry matter production was larger in medium-P soil than in low-P soil for lupine (+20%) and oat (+67%), while for corncockle it was the opposite, with a lower root dry matter production in the medium-P soil (−64%).

There was a significant effect of both the CC species and the soil P status on the P concentration in the shoot tissue, where corncockle had the highest concentration, and this was highest in the medium-P soil. Corncockle had the lowest C:P ratio (i.e., less C per unit P) in both shoot and root tissue, followed by lupine and then oat. In both roots and shoots, the highest C:P ratios were found in the low-P soil. Concerning C:N ratios, oat had the highest C:N ratio in the shoot tissue on both soils. This was also the case for the root tissue where it was followed by corncockle and then lupine. For all CC species, the C:N ratio in the root tissue was lower in the low-P soil than in the medium-P soil. The concentrations of C and N of the plant biomass are presented in the Supporting Information (Table S1). It should be noted that especially for root samples potential errors and variation related to contamination with soil particles cannot be ruled out. The smallness of the sample did not allow correction for this with additional analyses of sand/ash content.

TABLE 1 *p*-values for main effects and interactions for variables presented in figures and tables. Significant *p*-values (<0.05) are in bold

Variable	Main effects and interactions, *p*-values		
	Soil P status	Species	Soil P status × species
DM shoot	**<0.001**	**<0.001**	0.140
DM root	**<0.001**	**<0.001**	**0.006**
P conc shoot	**<0.001**	**<0.001**	**<0.001**
P conc root	**<0.001**	**<0.001**	**0.005**
C:N ratio shoot	**<0.001**	**<0.001**	0.321
C:N ratio root	**<0.001**	**<0.001**	0.169
C:P ratio shoot	**<0.001**	**<0.001**	0.377
C:P ratio root	**<0.001**	**<0.001**	0.995
Gram positive bacteria	**<0.001**	**0.008**	0.191
Gram negative bacteria	**<0.001**	**<0.001**	**<0.001**
Fungi PLFA	**<0.001**	0.746	0.921
Total microbial PLFA	**<0.001**	**<0.001**	**0.029**
AMF	**0.040**	**<0.001**	0.276
pH	**<0.001**	**<0.001**	0.824
Nitrate N	**<0.001**	**<0.001**	0.636
Extractable C	**<0.001**	0.466	0.343
Shoot P uptake	**<0.001**	**<0.001**	0.899
Root P uptake	**<0.001**	**<0.001**	**<0.001**
Phosphomonoesterase	**0.001**	**0.024**	**<0.001**
Phosphodiesterase	**<0.001**	0.822	0.104
B-glucosidase	**<0.001**	**<0.001**	0.303
N-acetyl-glucosaminidase	**<0.001**	**0.003**	0.376
P_{H2O}	**<0.001**	0.271	0.157
P_{resin}	**<0.001**	**0.003**	**0.042**

Corncockle and lupine shoots took up almost double the amount of P compared to oat. The uptake of P was significantly higher in the medium-P soil. In roots, lupine had the highest uptake of P of the CC species, and it was higher in the medium-P soil than in the low-P soil (Figure 1, Table 1). For all CC species, more P was allocated to shoots than to roots.

3.2 | C, N, and P content in soil microbial biomass

The C content of the soil microbial biomass after lupine was higher than after oat and corncockle. Furthermore, the effects of soil P status and CC were significant for C_{mic} (Table S2). The N content of soil microbial biomass did not vary between CCs, but was consistently higher in the medium-P soil compared to the low-P soil for all CC species. The P content of the soil microbial biomass was highest in the medium-P soil, and there was no significant effect of CC.

Lupine gave rise to the highest microbial C:N ratio (Table S2). There was a significant effect of soil P status for the C:P_{mic} ratio, but there was no significant effect of CC species (Table S2).

3.3 | Microbial community

The abundance of gram-positive and gram-negative bacteria was highest after lupine (Table 3). For fungal abundance, there was a significant effect of soil P status (Table 1), where the abundance was highest in the medium-P soil (Table 3). However, in the low-P soil, total microbial PLFA was significantly higher (21%) after growth of lupine compared to the no-crop reference treatment. The AMF abundance, estimated by the NLFA biomarker 16:1ω5, was significantly more abundant after the mycorrhiza-forming oat in both soils, and the highest amount of AMF biomass was found in the low-P soil after oat (Table 3).

3.4 | Enzyme activity

The activity of phosphomonoesterase increased significantly after lupine compared to no-crop, oat, and corncockle in the low-P soil, whereas there was no difference among CC treatments in the medium-P soil (Figure 2A). The effect of CC treatment on phosphodiesterase activity was not significant, whereas low-P status resulted in slightly but significantly higher phosphodiesterase activity compared to medium-P soil (Figure 2B, Table 1). The activity of β-glucosidase was significantly lower after oat when compared to the no-crop and corncockle treatments (Figure 2C, Table 1). On both soils, lupine had significantly less activity of N-acetyl-glucosaminidase than no-crop and corncockle (Figure 2D, Table 1).

3.5 | Soil pH, water- and resin-extractable P, and mineral N

After lupine, soil pH on both soils was significantly lower compared to all other treatments (Table 3). The CC treatments had no effect on P_{H2O}, but P_{H2O} was significantly higher (on average 1.4 mg P kg^{-1}) in medium-P soil compared to low-P soil (Figure 3). As for P_{H2O}, and as expected, P_{resin} was consistently higher in medium-P than in low-P soil, but in contrast to P_{H2O}, P_{resin} was significantly reduced after corncockle relative to all other treatments. Ammonium-N was not detected in any of the samples (data not shown). The nitrate-N concentration in the soil after all CC species was significantly lower than in the no-crop treatment, indicating that all CC species in both soils have emptied the soil for N to the same degree. The amount of extractable C was on average 72 mg C kg^{-1}, and there were no significant differences among CC treatments, suggesting that the available carbon pool did not vary with CC, but only with soil P status, where the amount of labile C was consistently higher in the low-P soil.

4 | DISCUSSION

The three CC species produced highly different amounts of biomass in both roots and shoots. Consequently, species-specific effects on

TABLE 2 Estimated dry matter production (DM) and C:N and C:P ratios in shoots and roots of the three CC species grown in two soils with low- or medium-P status. Numbers in parentheses are model 95% confidence intervals. Values followed by different capital letters show significant differences between low- and medium-P soil within individual CC species, while values followed by different lowercase letters indicate significant differences between CC species within each soil

	Soil P status	Oat		Corncockle		Lupine	
DM (g pot^{-1})							
Shoot	Low	2.9 (2.3–3.6)	b	2.4 (1.7–3.0)	b	7.1 (6.5–7.8)	a
	Medium	3.3 (2.6–3.9)	b	2.7 (2.1–3.4)	b	7.5 (6.8–8.1)	a
Root	Low	1.2 (0.8–1.7)	Bb	1.8 (1.3–2.2)	b	4.0 (3.5–4.4)	Ba
	Medium	2.0 (1.5–2.4)	Ab	1.1 (0.7–1.6)	c	4.8 (4.3–5.2)	Aa
P concentrations (mg P g^{-1})							
Shoot	Low	1.7 (1.3–2.1)	Ab	6.1 (5.8–6.5)	Ba	2.0 (1.6–2.4)	Ab
	Medium	2.1 (1.7–2.5)	Ab	8.4 (8.0–8.8)	Aa	2.2 (1.8–2.6)	Ab
Root	Low	1.4 (1.0–1.8)	Ac	2.8 (2.4–3.1)	Ba	2.1 (1.8–2.5)	Ab
	Medium	1.6 (1.2–2.0)	Ac	4.3 (3.9–4.6)	Aa	2.6 (2.2–2.9)	Ab
C:N ratio							
Shoot	Low	34 (30–38)	a	22 (18–27)	b	17 (13–21)	b
	Medium	35 (31–39)	a	23 (19–27)	b	17 (13–21)	b
Root	Low	43 (40–46)	Ba	36 (33–39)	Bb	17 (14–19)	Bc
	Medium	46 (43–49)	Aa	39 (36–42)	Ab	20 (17–22)	Ac
C:P ratio							
Shoot	Low	249 (233–264)	Aa	37 (51–83)	Ac	221 (205–237)	Ab
	Medium	221 (205–237)	Ba	39 (23–55)	Bc	193 (178–209)	Bb
Root	Low	289 (276–303)	Aa	122 (108–135)	Ac	181 (168–195)	Ab
	Medium	261 (247–274)	Ba	93 (79–106)	Bc	153 (139–166)	Bb

the indices for plant-available P and soil microbial properties can be expected. In accordance with our hypothesis and in agreement with other studies (Nuruzzaman et al., 2005; Wen et al., 2019), shoot biomass production was higher in the medium-P soil than in the low-P soil, which indicates P limitation of plant growth in the low-P soil.

4.1 | CC growth, nutrient uptake, and partitioning between shoots and roots

The quality of the CC biomass expressed by the C:N and C:P ratios plays a major role for mineralization and effects on nutrient dynamics in the soil (Nguyen & Marschner, 2017). Lupine and corncockle had

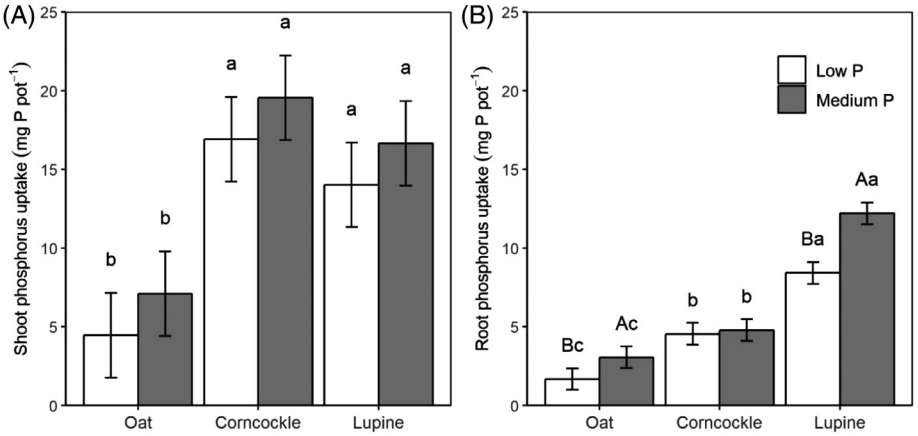


FIGURE 1 Shoot and root P uptake (mg P pot^{-1}) for the three CC species in soil with low or medium soil P status. Error bars show model 95% confidence intervals. Different capital letters on columns indicate significant differences between low- and medium-P soils within individual CC species, and columns with different lowercase letters show significant differences between CC species within each soil

TABLE 3 Estimated amount of gram-positive and gram-negative bacteria, fungi PLFAs, total microbial PLFA, AMF (NLFA biomarker 16:1ω5), pH ($CaCl_2$), nitrate-N, and extractable C content after growth of CC and the unplanted treatment in either low- or medium-P soil. Model 95% confidence intervals shown in parentheses. Values followed by different capital letters show significant differences between low- and medium-P soil within individual CC species, while values followed by different lowercase letters indicate significant differences between CC species within each soil

	Soil P status	No crop		Oat		Corncockle		Lupine	
Gram pos bacteria PLFA	Low	5.88 (5.58–6.18)	b	6.23 (5.93–6.54)	ab	6.08 (5.78–6.39)	ab	6.56 (6.25–6.86)	a
(nmol g^{-1})	Medium	5.89 (5.59–6.20)	b	6.24 (5.94–6.55)	ab	6.10 (5.79–5.78)	ab	6.57 (6.27–6.87)	a
Gram neg bacteria PLFA	Low	1.09 (1.02–1.17)	ab	1.08 (1.01–1.15)	ab	1.12 (1.04–1.19)	b	1.57 (1.50–1.64)	Aa
(nmol g^{-1})	Medium	1.13 (1.06–1.21)	b	1.17 (1.10–1.24)	b	1.09 (1.02–1.17)	b	1.23 (1.16–1.31)	Ba
Fungi PLFA	Low	0.85 (0.60–1.11)		0.83 (0.58–1.09)		0.69 (0.44–0.94)		0.80 (0.55–1.06)	
(nmol g^{-1})	Medium	0.88 (0.63–1.13)		0.86 (0.61–1.11)		0.71 (0.46–0.97)		0.83 (0.58–1.08)	
Total microbial PLFA	Low	10.8 (10.1–11.5)	b	11.1 (10.4–11.8)	b	11.1 (10.4.11.8)	b	13.1 (12.4–13.8)	a
(nmol g^{-1})	Medium	11.0 (10.3–11.7)		11.8 (11.1–12.5)		11.1 (10.4–11.8)		11.7 (11.0–12.4)	
AMF NLFA	Low	0.87 (0.42–1.33)	b	5.25 (2.51–7.99)	a	0.99 (0.47–1.51)	b	1.26 (0.60–1.91)	b
(nmol g^{-1})	Medium	1.03 (0.49–1.56)		2.57 (1.23–3.92)		1.01 (0.48–1.54)		0.94 (0.45–1.44)	
pH ($CaCl_2$)	Low	6.4 (6.2–6.5)	a	6.4 (6.2–6.5)	a	6.2 (6.1–6.4)	a	5.9 (5.8–6.0)	b
	Medium	6.5 (6.3–6.6)	a	6.5 (6.3–6.6)	a	6.3 (6.2–6.4)	a	6.0 (5.8–6.1)	b
Nitrate-N	Low	18.7 (18.2–19.2)	a	0.6 (0.1–1.0)	b	0.5 (0.0–1.0)	b	0.2 (0–0.6)	b
(mg N kg^{-1})	Medium	18.7 (18.3–19.2)	a	0.6 (0.1–1.0)	b	0.5 (0.0–1.0)	b	0.2 (0.0–0.6)	b
Extractable C	Low	67 (51–84)		78 (62–95)		77 (61–93)		83 (67–99)	
(mg C kg^{-1})	Medium	59 (43–75)		70 (54–86)		68 (52–85)		74 (58–90)	

the lowest C:N ratio, which could indicate that N from their residues will mineralize faster than that from oat (Janssen, 1996; Li et al., 2020). However, there is no consensus on this in the literature. Jensen et al. (2005) did not observe this relationship between C:N ratios and N mineralization in a test of 37 different plant species. In case lower C:N and C:P ratios will lead to faster mineralization, it can be expected that lupine and corncockle shoots will decompose faster than oat, and that lupine roots will decompose faster than oat and corncockle roots, which can have implications for the P nutrition of the following crop. This, however, remains to be studied together with a further characterization of the plant material and its structural components (lignin and cellulose).

Corncockle stood out from the two other CCs with the lowest root biomass. Despite this, corncockle took up as much P as lupine, and had a significantly higher P uptake than oat. It also had a lower C:P ratio in the plant tissue than the other CCs. This will likely have an impact on the release of P through mineralization of plant material (Erinle & Marschner, 2020), because the release of P from organic sources is typically driven by the microbial need for C (Heuck et al., 2015). Hence, plant residues with high P concentrations can possibly have a positive effect on plant-available P in soil (Damon et al., 2014). Some of the P in plant tissue, especially in corncockle, might be stored as a reserve in the form of inorganic P in the vacuoles (Bieleski, 1973; Chapin III, 1980). All CCs responded with a higher root C:P ratio in the roots in low-P soils compared to the medium-P soil.

4.2 | Modifications of soil microbial community and enzyme activity

We expected that CCs would stimulate soil enzymes by the following mechanisms: CCs provide substrates for growth of soil microorganisms that excrete extracellular enzymes in C-, N-, and P-cycling. The excretion of enzymes by bacteria and fungi might be either proportional to their growth or overproportional (Waldrop et al., 2000). CC growth did not result in increased ß-glucosidase and N-acetyl-glucosaminidase activities compared to bare soil. In a recently published meta-analysis on the effect of CC on the soil microbiome, Kim et al. (2020) found, on the basis of 60 field and greenhouse studies, that the microbial activity (measured as the activity of β-glucosidase and phosphatase and soil respiration) increased after CC, on average by 22%. In contrast, Rankoth et al. (2019) found that CCs did not affect ß-glucosidase activities in a two-year on-farm study, and we found decreased β-glucosidase activity after CC growth. We are aware that any growth of CC enhances C input by root exudates and rhizodeposits. Since low availability of substrates in the rhizosphere of CC can be excluded as the reason for lower ß-glucosidase and N-acetyl- glucosaminidase, we speculate that differences in moisture content between bare soils and planted soils might explain our results. Moisture content in soils is an important driver of production, expression, and turnover of different soil enzymes (Müller et al., 2016; Preusser et al., 2017). In our study, we watered the plants according to need, but never more than once a day,

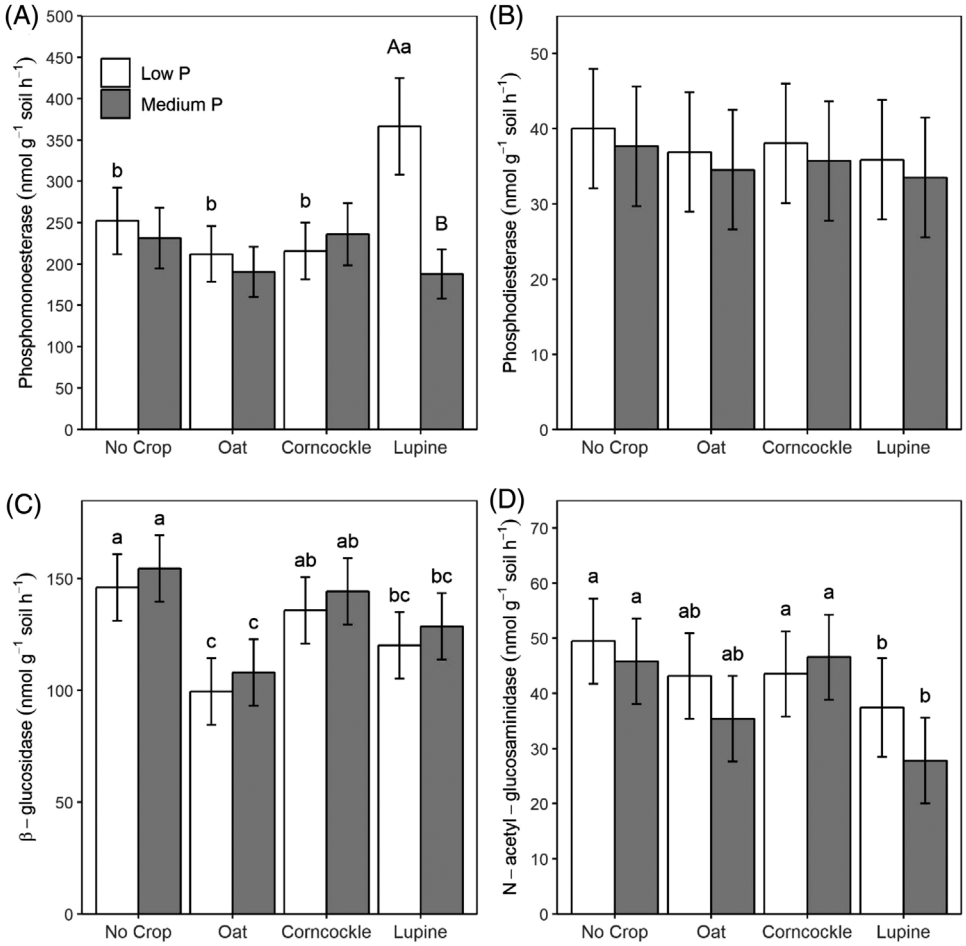


FIGURE 2 (A) Phosphomonoesterase, (B) phosphodiesterase, (C) β-glucosidase, and (D) N-acetyl-glucosaminidase after growth of CC and in unplanted soil with low or medium soil P status. Error bars show model 95% confidence intervals. Capital letters show significant differences between low- and medium-P soils within individual CC species and the lowercase letters show significant differences between CC species within each soil

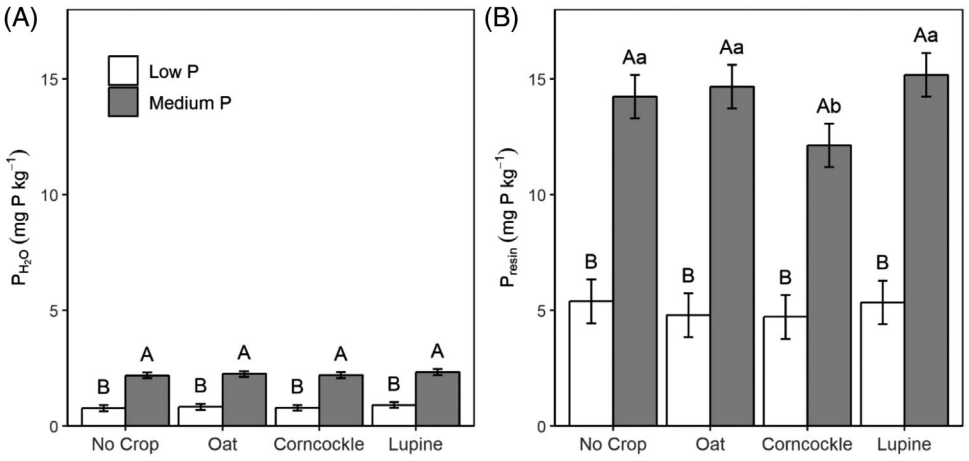


FIGURE 3 (A) P_{H2O} and (B) P_{resin} after growth of CC and in unplanted soil with low or medium soil P status. Error bars show model 95% confidence intervals. Capital letters show significant differences between low- and medium-P soils within individual CC species and the lowercase letters show significant differences between CC species within each soil

which may have led to unfavorably dry conditions, especially toward the end of the experiment where both transpiration and temperatures increased. During the experiment, lupine especially transpired large amounts of water, making the soil fairly dry (down to 5% water at the driest). These drying–rewetting cycles might have affected the physical, chemical, and microbial properties of the soil, which in turn might have affected the P retention on soil surfaces and the turnover of P in the soil (Gao et al., 2020). Due to the higher biomass production of lupine, it is plausible that such drying–rewetting effects were more pronounced in the lupine soil. The high microbial C:N and C:P ratios in the lupine treatment might be connected to nutrient release from the microbial biomass at the moment of sampling. Furthermore, both microbial and plant-derived soil enzymes are stabilized in organo–mineral complexes under dry conditions (Datta et al., 2017; Kandeler et al., 2019), which can reduce the contact between the adsorbed extracellular enzymes and their corresponding substrates, leading to lower potential enzyme activities (Evans et al., 2016).

After lupine growth, we observed a lower activity of the N-cycling enzyme, N-acetyl-glucosaminidase, compared to the other treatments. This enzyme is mainly derived from fungi; however, there was no difference in fungi abundance after lupine compared to the no-crop treatment, suggesting that the fungi in the lupine treatment produced less N-acetyl-glucosaminidase. In addition, the content of mineral N in soil was generally very low at the end of the experiment, which showed that all three CCs had effectively taken up N from soil.

The most obvious result was that the low-P soil induced an increase of phosphomonoesterase activity in the lupine treatment. Hallama et al. (2019) showed that the increased phosphomonoesterase activity under CC treatments was accompanied by an increased capacity of the microbial community to use specific phosphate-bearing substrates, such as glycerolphosphate and glucose-1-phosphate. Since phosphomonoesterases are specifically produced under P limiting conditions (Wasaki et al., 2005), the general stimulation of microbial growth by root exudates as well as the presence of phosphate-bearing substrates might be the reason for our result. Although not significant, our results showed a trend for higher enzyme/C_{mic} ratios in the low-P soil, which might be explained by the need of soil microorganisms to produce more enzymes to satisfy their nutrient demand under P limitations (Table S3). It is not possible to fully identify the main producers of phosphomonoesterase in soils (Godin et al., 2019), since methods to estimate the group-specific enzyme activities are not available. Future analyses using metagenomic techniques might clarify the relative contribution of fungi, bacteria, and roots to the overall activity of this enzyme. However, the higher ratio between the activity of phosphomonoesterase and total microbial PLFA for lupine grown in the low-P soil compared to the rather similar ratios of the bare soil, corncockle, and oat treatments suggests that the microbes under lupine produced relatively more phosphomonoesterase than under corncockle and oat. Even though the ratio indicated larger specific activity by microbes in the low-P soil with lupine, this does not rule out that lupine roots also exuded phosphomonoesterase (Chavarria et al., 2016). In agreement with our results, Nuruzzaman et al. (2006) found the highest increase in phosphatase activity in soil with a lower P status. This indicates that in the low-P soil, the lupine managed to increase the activity of phosphatase to ensure a sufficient supply of P. Wasaki et al. (2018) demonstrated that lupine roots can react to P deficiency by the release of citrate and phosphomonoesterase, especially from their cluster root structures. We did not observe typical cluster root structures in our experiment, but we did observe a distinctive rhizosheath around the roots, probably due to a profusion of small root hairs of *L. angustifolius*. This layer may be similar to the cluster-like structures on the entire root system observed for *L. angustifolius* by Hocking and Jeffery (2004). Furthermore, lupine in our study clearly stimulated the microbial community compared to the two other species and the control by increasing total microbial PLFA and the abundance of gram-negative bacteria, especially in the low-P soil.

Oat, being the only mycorrhiza-forming species we tested, increased the abundance of the AMF biomarker NLFA 16:1ω5, especially in the low-P soil, which agrees well with the findings of Williams et al. (2017). In soils with higher P availability, plants allocate less C to AMF (Williams et al., 2017). For main crops relying on mycorrhiza for P supply in a low-P soil, the capacity of a CC to increase the AMF abundance, and hence the inoculation potential for the main crop, may potentially be more important than the amount of P returned to the soil with the oat residues (Hallama et al., 2019). Murrell et al. (2020) found that CCs forming AMF enhance the P uptake of a following maize crop compared to non-AMF CC species.

Corncockle did not affect the microbial community structure and activity in ways directly related to microbial turnover of organic P, as did lupine and oat. For corncockle, a potential positive effect on main crop P nutrition would be if they could make use of the large amounts of P returned to the soil with the CC biomass.

4.3 | Modifications in soil water- and resin-extractable P

In spite of the different amounts of P taken up by the CC, we could not detect any changes in P_{H2O} in soil compared to bare soil after the growth of the three CC species. P_{H2O} represents a small dynamic proportion of the soil P, which is, or can very easily be, released to the soil solution (Hinsinger, 2001). This fraction is buffered by the larger fraction of inorganic P retained on soil particle surfaces (Frossard et al., 2000). This buffering of P_{H2O} may explain why we do not detect changes in this pool. For P_{resin}, representing a larger pool of inorganic P acting as the immediate buffer for P_{H2O}, we found that corncockle significantly reduced this pool of P in the medium-P soil compared to the other two CC species and bare soil. The fact that only corncockle reduced P_{resin} in the medium-P soil, while lupine that took up almost equal amounts of P did not, may suggest that corncockle depleted the P_{resin} pool, while lupine made more use of other soil P pools (Lambers et al., 2013). Such pools are present in the soils used in this study. They had total P contents of 330 and 420 mg P kg^{-1} and oxalate-extractable P contents of 260 and 360 mg kg^{-1} in the low- and medium-P soils, respectively, as determined in Azeez et al. (2020). Furthermore, according to Schoumans (2000), oxalate-extractable P

in noncalcareous soils represents the entire pool of P sorbed to soil surfaces acting as a buffer for the more readily exchangeable P pools measured in this study. From a visual judgement, corncockle had a finer and more extensive root system than the other CCs, perfect for exploring the soil volume for inorganic P sorbed on soil surfaces. Corncockle probably made an efficient use of such P pools, because its fine roots explored the soil volume more thoroughly than the two other species. It is worth remembering when looking at the P_{resin} result that corncockle in the medium-P soil actually had the lowest amount of root DM, both compared to the other species and to corncockle grown on low-P soil. That corncockle relied on other P uptake mechanisms than oat and lupine corresponds well with the findings of Wen et al. (2019), that species with thinner roots have less AMF and release fewer root exudates. Likewise, Honvault et al. (2021), who studied 10 morphological and physiological traits of 13 different plant species involved in P acquisition, found that thick roots in general released more carboxylates and generated more phosphatase activity than finer roots. This correlates well with our results, where lupine had much thicker roots than corncockle and oat. Lupine was also the only species in our study that increased the activity of phosphomonoesterase, which suggests that lupine relied more on the organically bound P for its supply of P than the two other species as suggested by Nuruzzaman et al. (2006).

4.4 | Perspectives and practical implications

This study evaluated the effects of three CC species on important factors for P turnover and P availability in soils right after termination of the CC. The clear differences between the three CC species in nutrient uptake and the clear differences after growth on soil microbial activity and P_{resin} observed in this study highlight that it might be possible to select the best species for each cropping system to improve the use of soil P in the subsequent crop. Important next steps are to evaluate if the growth of certain CC species also proves beneficial for the P supply of the subsequent main crop in different cropping systems, as it has been done in a pot experiment for these specific soils and CC species (Christensen et al., 2021). It is important to include field trials in future studies of CC effects on P turnover and P supply to the main crop.

Furthermore, our results indicate that the impact of CC on the soil P dynamics varies with soil P status. Testing on soils with a higher soil P status would be important if the benefits of CC are to be promoted as tools for improving use of P in soils with high P status.

5 | CONCLUSIONS

This study demonstrated that three different CC species affected the nutrient dynamics, soil microbial structure, and community differently.

Oat increased the amount of AMF in the soil after its growth. Corncockle effectively mined the soil for resin-extractable P and the plant tissue had a remarkably high P concentration. Lupine produced large amounts of biomass and increased the total amount of microbes in the soil. Therefore, the potential benefits of selecting CC species used for nitrate catching, that additionally improve the use of soil P in the following main crop, deserve further investigation.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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