Received: 12 February 2021 | Accepted: 22 August 2021

DOI: 10.1002/jpln.202100046

REGULAR ARTICLE

Effect of soil P status on barley growth, P uptake, and soil microbial properties after incorporation of cover crop shoot and root residues

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Abstract

Background: Cover crops (CC) introduced to reduce leaching of nitrate may also improve soil microbial properties and phosphorus (P) uptake in crops that follow. These effects may depend on soil P status and the quality of the CC residues.

Aim: Our aim was to quantify the more persistent effects of incorporation of residues of three different CC species on barley growth and P uptake and whether any response in growth and P uptake was related to soil P status and changes in soil microbial properties.

Methods: We examined the impact of soil P status, CC species, and residue fraction (shoots and/or roots) on the growth of winter barley (*Hordeum vulgare*) and soil microbial properties. Shoot and root residues from three contrasting CC (oats, *Avena sativa*; corncockle, *Agrostemma githago*; lupine, *Lupinus angustifolius*) were incubated for 2 months in soil with low and medium P status. Dry matter yield and P offtake characterized effects of CC residues on barley while phospholipid fatty acids (PLFAs), neutral lipid fatty acids (NLFAs), and enzyme activity characterized soil microbial properties after barley harvest.

Results: Effects of shoot residues on barley yield and P offtake appeared neutral or positive and not affected by soil P status, while the effect of root residues appeared negative for medium P soil and neutral for low P soil. With lupine shoot biomass, however, barley yield and P offtake increased in both soils. Neither CC treatments nor soil P status affected the microbial community composition (bacterial and fungal specific PLFAs). Lupine roots increased phosphomonoesterase, and lupine residues generally stimulated microbial abundance (total microbial PLFAs) compared to other CC treatments.

Conclusion: We conclude that the effect of CC residues on the following crop and soil microorganisms depends on soil P status and that lupine had the largest positive impact on barley growth.

KEYWORDS
corncockle, green manure, lupine, phospholipid fatty acid, neutral lipid fatty acid, oat

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1 | INTRODUCTION

Under temperate climatic conditions, cover crops (CC) are often used to retain mobile forms of plant nutrients, such as nitrate and sulfate, from being lost by leaching (Aronsson et al., 2016; Eriksen & Thorup-Kristensen, 2002). Incorporation of CC in their vegetative phase may provide additional benefits to soil quality (Blanco-Canqui et al., 2015; Thorup-Kristensen et al., 2003), including improved soil structure and enhanced soil microbial and faunal activity. Further, nutrients temporarily retained in CC residues may become available for the subsequent main crop.

The mobility of P in soil is low, and leaching of P is most often unaffected by CC (Norberg & Aronsson, 2020), whereas CC may play an important role in reducing losses of P by erosion and surface flow from sloping areas (Aronsson et al., 2016). In addition, CC residues may enhance both yields and P uptake in subsequent crops (Hallama et al., 2019). However, effects of CC on the subsequent crop vary widely and appear to depend on CC species, maincrop type, and soil P status (Cavigelli & Thien, 2003; Damon et al., 2014; Eichler-Löbermann et al., 2009; Hallama et al., 2019; Nuruzzaman et al., 2005).

An adequate supply of P is critical for early root development and thereby for crop growth and final yields (Grant et al., 2001; Pedersen et al., 2021). For CC incorporated in the vegetative phase, water-soluble inorganic P may account for 60–80% of their total P content whereby CC residues may become an important source of readily plant-available P for the succeeding crop (Damon et al., 2014). However, inorganic P may also be sorbed onto soil components or immobilized in microbial biomass (Bünemann et al., 2012) that becomes stimulated by the input of available carbon (C) from the plant residues.

Sorption of inorganic P links closely to soil P status, while microbial activity links to the accessibility of incorporated plant residues (Kim et al., 2020). There is evidence of CC species as well as CC part (shoot or root) affecting the amount of P available to the main crop differently (Alamgir et al., 2012; Cavigelli & Thien, 2003; Fuller et al., 1956; J. Liu et al., 2015). Hu et al. (2018) observed that net N mineralization was faster for shoots than for roots; we expect a similar pattern for P release from CC shoots and roots. The microbial activity fueled by inputs of C substrates affects soil P turnover not just by immobilization of inorganic P but also by mineralization of organic P accumulated in the microbial biomass or released from soil organic matter by extracellular microbial enzymes. Incorporation of CC residues may therefore accomplish a net loss or a net gain in the pool of P available to the following crop. Further, interactions between the soil microbial community and plant roots may induce changes in the rhizosphere of the growing main crop, for example, by affecting soil pH, and thereby releasing P sorbed to soil minerals (Hinsinger, 2001). To increase the use-efficiency of plant nutrients in intensive cropping systems, there is a need to quantify any additional benefits of CC and to improve our understanding of the role of plant–microbial interactions for nutrient cycling in systems with different soil P status

Here we study the more persistent effects of incorporation of the shoot and root residues of three CC species with contrasting growth characteristics and differing strategies of P uptake on a subsequent crop of winter barley (*Hordeum vulgare*) as a test crop. We selected oats (*Avena sativa*), a common CC in Denmark, because of its symbiosis with mycorrhiza fungi. Corncockle (*Agrostemma githago*) has shown a high concentration of P in the plant tissue in a previous field experiment (unpublished), and lupine (*Lupinus angustifolius*) was included due to its ability to access immobile soil P (Lambers et al., 2013). We used a coarse sandy soil from a long-term field experiment where different P fertilization histories provided soils with widely different P status. We envisioned a higher availability to the barley of P incorporated with shoot than with root residues, and that a beneficial effect of CC incorporation would be higher in soil with low P than with high P status (Hallama et al., 2019). Dry matter yield and P offtake characterized the effect of CC residues on barley, while the determination of phospholipid fatty acids (PLFAs), neutral lipid fatty acids (NLFA), and enzyme activity characterized effects on the soil microbial community structure and activity after barley harvest. We expected different CC to modify the abundance, composition, and function of soil microorganisms differently, leading to differences in the release of P from the residues.

2 | MATERIALS AND METHODS

2.1 | Soils

The soil was retrieved from a long-term agricultural field experiment located at Jyndevad Experimental Station, Denmark (54°54′ N, 9°07′ E). The experiment, initiated in 1942–1944, combines four rates of lime with two rates of mineral P fertilizer and cropped mainly with spring barley since then. The textural composition of the soil is 4% clay (< 2 μm), 4% silt (2–20 μm), 17% fine sand (20–200 μm), 75% coarse sand (200–2000 μm), and 1.3% C at 0–25 cm depth. The site classifies as Orthic Haplohumod according to U.S. Soil taxonomy (Nielsen & Møberg, 1985; Soil Survey Staff, 1975) with mean annual precipitation and temperature of 870 mm and 7.9°C, respectively. Azeez et al. (2020) and Rubæk et al. (1998) provide further details on the experiment.

In March 2018, we collected 340 kg soil from 0 to 20 cm depth in each of two treatments: low P soil with no fertilizer P applied since 1944 and medium P soil with 15.6 kg fertilizer P ha^{-1} added annually. Both treatments received an initial dose of 156 kg P ha^{-1} in 1944 and were then subject to the same lime treatment (8 Mg ha^{-1} applied every 5–9 years). Low and medium P soils were similar in pH (6.6). Olsen-P values were 11 and 21 mg P kg^{-1}, respectively, corresponding to low and medium P fertility status in Danish recommendation schemes for P fertilization (Jordan-Meille et al., 2012). After mixing and sieving (< 1 cm), we packed field moist soil to a bulk density of 1.3 g dry soil cm^{-3} in PVC pots (inner diameter 11 cm, height 40 cm). In total, we established 44 pots with low P soil and another 44 with medium P soil.

TABLE 1 Characteristics of oats, corncockle, and lupine shoot biomass added to low P and medium P soil

	Soil P status	Corncockle	Lupine	Oats
DM ($g\ pot^{-1}$)	Low	2.4	7.1	2.9
	Medium	2.7	7.5	3.3
P concentration in shoot($mg\ P\ g^{-1}$)	Low	6.1	2.0	1.7
	Medium	8.4	2.2	2.1
P in shoot ($mg\ P\ pot^{-1}$)	Low	1.7	1.4	0.4
	Medium	2.0	1.7	0.7
N concentration in shoot($mg\ N\ g^{-1}$)	Low	16.6	26.0	13.2
	Medium	16.5	25.9	13.1
N in shoot ($mg\ N\ pot^{-1}$)	Low	39.4	186.3	36.2
	Medium	45.1	192.0	41.8
C:N ratio	Low	22	17	34
	Medium	23	17	35
C:P ratio	Low	37	221	249
	Medium	39	193	221

2.2 | Growth of cover crops

Three CC species were sown on May 3, 2018: oats, corncockle, and lupine. All CCs grew in both low P and medium P soil, each with eight replicates (total 48 pots). For each soil, 12 replicate pots were without CC (bare soil; total 24 pots). The pots were randomly placed in four blocks on racks in an outdoor growth facility. Seven seeds were sown in each pot with CC, and 6 days after sowing (DAS) the number of plants was reduced to four for oats and corncockle and three for lupine. The pots received demineralized water every 1–3 days to maintain 60% water-holding capacity (WHC) and were exposed to ambient weather conditions, including mild rain. During heavy rains, a roof covered the pots. At 21 DAS, irrigation increased to 70% of WHC. The mean temperature during the growth period was 17°C. Plant macronutrients other than P were applied 12 DAS at rates corresponding to ($g\ m^{-2}$) 3.0 N (NH_4NO_3), 12.5 K (K_2SO_4), 10.0 Ca ($CaCl_2$), and 5.0 Mg ($MgCl_2$). We also added a micronutrient solution corresponding to ($g\ m^{-2}$) 0.245 Mn ($MnSO_4$), 0.196 Zn ($ZnSO_4$), 0.054 B (H_3BO_3), 0.196 Cu ($CuSO_4$) 0.005 Co ($CoCl_2$), and 0.025 Mo ($NaMoO_4$).

2.3 | Soil incubation of CC residues

The growth of all CCs was terminated on 13 June (40 DAS) by cutting shoots at the soil surface. Table 1 presents the general characteristics of the CC shoot materials. After cutting the shoot material into 1 cm pieces, the following incubations were established: (1) shoot material from CCs incorporated into pots with bare soil, (2) shoot material from CCs incorporated into the soil in which they were grown, and (3) pots with no shoot material incorporated. Thus, the pots used for incubations (2) and (3) encompassed the root system left in the soil from the given CC. Pots for reference were kept bare, and no plant residues were added to these pots (16 pots). We introduced the shoot material into the soil by pushing the upper 10 cm out of the pot with a hydraulic piston, mixing the soil with shoot material, and then returning the amended soil back into the pot. Pots with bare soil and no CC residues, and pots with roots only also passed this procedure.

2.4 | Growth of winter barley

Winter barley (*Hordeum vulgare*) was sown in all pots on 10 July (28 days after the termination of CCs) using seven seeds per pot. After germination, surplus plants were removed, leaving four plants in each pot. At this stage, all pots received a nutrient amendment similar to that used for the growth of CC. Further, half of the reference pots were kept in bare soil until now received mineral P fertilizer corresponding to 2 g P m^{-2} (termed: P fer.), while the other half remained unfertilized (termed: no P). This involved four replicates for low P and four for medium P soils. On 27 July, all pots received mineral N fertilizer corresponding to 3 g N m^{-2}.

After counting the number of tillers on 28 August (49 DAS; Zadoks' growth stage 31–32), we cut the shoot material of barley plants at the soil surface and dried it at 60°C for 48 h for the determination of dry matter (DM) yield and P content. Soil from each pot was passed through a 4-mm sieve, and roots were removed. The sieved soil was mixed and subsampled for chemical analyses, including DM determination (105°C for 24 h). The remaining soil was stored at −18°C for microbial analyses. All results of soil analyses are presented on a dry weight basis.

2.5 | Chemical analyses

For the determination of P concentration, barley shoot subsamples were ball milled and 300 mg digested under pressure with 3 mL H_2O_2 (9.7 M) and 6 mL HNO_3 (14.3 M) in a microwave oven for 45 min (Anton Paar GmbH, Graz, Austria). The P content in the digestate was analyzed according to N. Liu et al. (2013) using inductively coupled plasma–optical emission spectroscopy (iCAP 6000, Thermo Fisher, Waltham, MA, USA).

Soil pH was measured using a pH electrode on 5 g moist soil in a 0.01 M $CaCl_2$ solution. Water-extractable P (P_{H2O}) was determined on 5 g moist sieved (< 2 mm) soil shaken for 17 h with 50 mL demineralized water. The soil suspension was centrifuged for 10 min at 1831 × *g* ,and the P content in the supernatant was determined colorimetrically according to ISO 6878 (ISO, 2004).

2.6 | Microbial community structure

The structure of the microbial community was determined by the extraction of PLFAs and NLFAs and assigning them by biomarkers to specific groups of organisms. The PLFAs and NLFAs were extracted from 4 g of frozen moist soil over a period of 2 h using the reagent

of Bligh and Dyer (1959) (citrate buffer: chloroform: methanol in the volume ratio of 1:2:0.8). Citrate buffer and chloroform were added for phase separation, the organic phase then being evaporated under N_2 flow. The lipids were separated on silica columns. NLFAs were extracted by flushing with chloroform and PLFAs by flushing with methanol. Methanolysis of PLFAs and NLFAs was done by the addition of methanol–toluol–solution and 0.2 M methanolic KOH. After incubation for 15 min at 37°C, the 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 used as an indicator for abundance of Gram-positive (i15:0, a15:0, i16:0 and i17:0) and Gram-negative bacteria (cy17:0 and cy19:0) and fungi (18:2ω6). The total microbial PLFA was calculated from the sum of biomarkers for Gram-positive bacteria, Gram-negative bacteria, fungi, 16:1ω5, 15:0, and 16:1ω7. The NLFA biomarker 16:1ω5 estimated mycorrhiza biomass (Frostegård & Bååth, 1996).

2.7 | Enzyme activity

The activity of the enzymes phosphomonoesterase, phosphodiesterase, beta-glucosidase, and *N*-acetyl-glucosaminidase was determined using the fluorimetric substrate assay described by Marx et al. (2001) and modified by Poll et al. (2006). All substrates contained the fluorescent compound 4-methylumbelliferone. One gram of frozen field moist soil was dispersed by ultrasonication (50 J s^{-1}, UP200S, Hielscher) 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 the florigenic substrate, and 50 µL of 0.1 M MES buffer [2-(*N*-orpholino)ethanesulfonic acid, pH 6.1]. For each enzyme and soil, standards were prepared to create specific standard curves. The standards consisted of a 50-µL soil solution with combinations of buffer and florigenic substrate to create increasing concentrations of the working solution. All microplates were placed in an incubator at 30°C, and the plates were read on a microplate reader (FLX 800, Microplate Fluorescence Reader, Bio-Tek Instruments Inc., USA) at 360/460 nm after 0, 30, 60, 120, and 180 min of incubation. For each soil sample, all enzyme analyses included three analytical replicates. Enzyme activity was determined by relating the linear slope of emitted fluorescence with corresponding standards (Poll et al., 2006).

2.8 | Calculations and statistical analyses

The uptake of P was calculated by multiplying the P concentration with the DM yield of barley. The data were analyzed using three-way ANOVA on linear mixed models with soil P status, CC species, and CC residues as fixed effects and block as a random effect using the R package *lme4* (Bates et al., 2014). All models relied on normal distributions, except the model for P_{H2O} for which we applied an identity link function with a gamma distribution. Pairwise comparisons were conducted using Tukey's post hoc tests ($p < 0.05$) implemented in the CLD function in the *emmeans* R package. Reference treatments were not included in the analysis of variance (ANOVA) and Tukey's tests. Instead, Dunnett's test ($p < 0.05$) was performed to identify treatment differences from the no P reference treatment (without any P application). Data presented in tables and figures are means and standard errors.

3 | RESULTS

Shoot DM yield and P uptake were significantly higher in medium P than in low P soil (Figure 1A, Table 2). Interestingly, DM yield did not respond to the addition of P fertilizer in reference treatments without CC residue amendments; this was true for low P as well as medium P soil (Figure 1A, Table 3). Generally, the presence of CC root residues reduced yields of barley grown in the medium P soil, whereas the lower yields retrieved in the low P soil were almost unaffected by residue incorporation. For soil with oats or corncockle root residues, yields did not differ between medium P and low P soils. The quantitatively most important effect of CC residues was the increase in yields of barley grown on medium P soil amended with corncockle and lupine shoot residues, where the yields after lupine shoot residues were significantly higher than yields obtained on reference treatments without CC residue amendments. Yields of barley grown on the low P soil showed a similar trend. Compared to medium P soil with shoot residues, incorporation of root + shoot residues generally reduced barley yields. However, barley yield on the medium P soil remained higher for lupine compared with corncockle and oats in the shoot + root treatments. For the low P soil, the incorporation of root + shoots residues showed barley yield similar to that obtained on the reference treatments.

The effect of CC residues on P uptake in barley followed a pattern very similar to that seen for DM yields (Figure 1B, Table 3), reflecting that the concentration of P in the harvested barley biomass was little affected by CC residues (data not shown). Furthermore, the number of tillers counted just before barley harvest on soils with CC residues was not significantly different from the number of tillers observed for the treatments without residues (Figure 1C, Table 3). There was a significant interaction between soil P status and CC species (Table 2). Especially lupine residues gave rise to more tillers in medium P soil than in the low P soil. This was also true for the number of barley tillers after amendment with corncockle shoots, but not with oats shoots.

Figure 1D shows the concentration of water-extractable P (P_{H2O}) in soil after barley harvest. The addition of fertilizer P increased P_{H2O} significantly in low P as well as medium P soil (Table 3). For all CC residue treatments, P_{H2O} was higher in medium P than in low P soil. Generally, incorporation of CC residues did not change P_{H2O} compared to the reference treatment without fertilizer P addition (no P), the only exception being slightly higher values for pots with corncockle root + shoot residues. Soil P status, CC species, and residue part all significantly affected P_{H2O} with no significant interactions between the variables (Table 2).

Soil P status did not significantly affect the activity of phosphomonoesterase, but CC species and residue part did (Table 2). The activity of phosphomonoesterase was in general several folds higher than

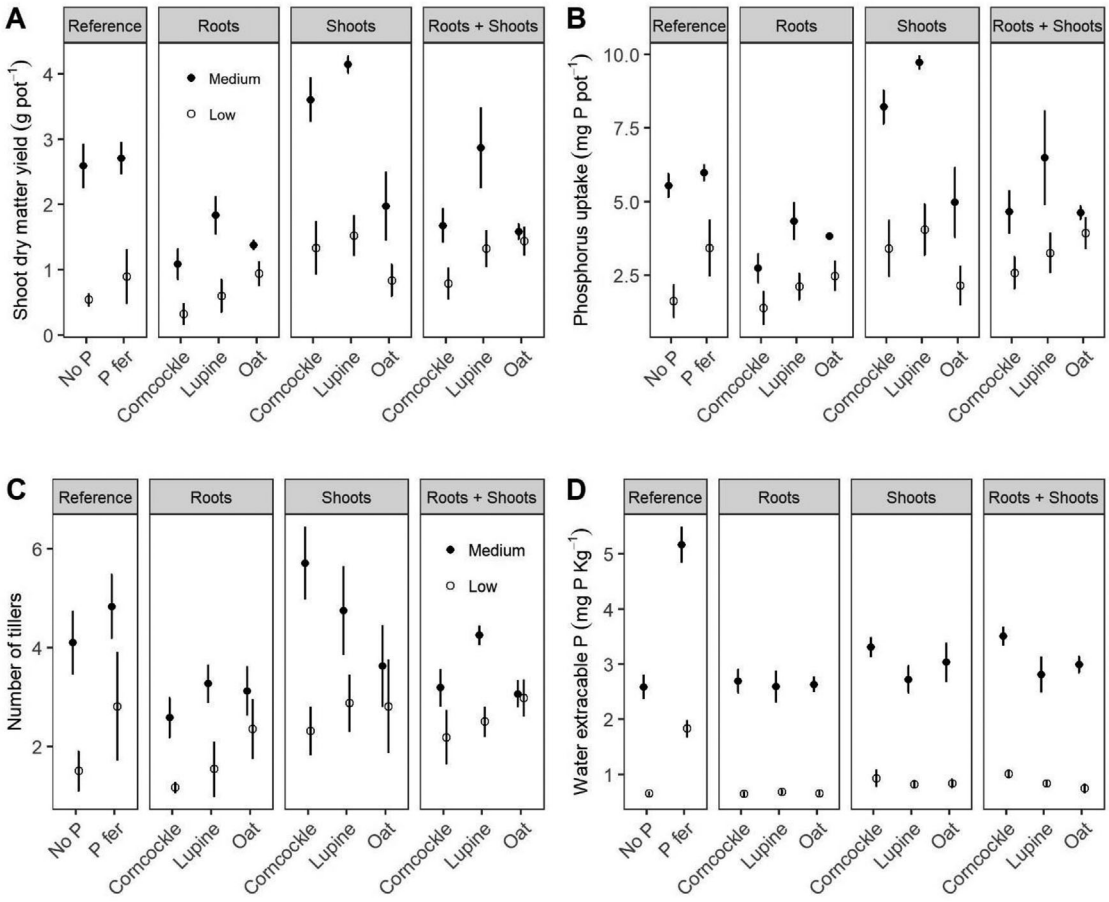


FIGURE 1 Barley DM yield (A), P uptake (B), number of tillers (C), and water-extractable P (D) for treatments with cover crop residues and reference treatments. Filled circles show results from the medium P soil and open circles show results from the low P soil. Points are means, and error bars indicate standard errors ($n = 4$)

that of phosphodiesterase. For phosphodiesterase, soil P status, CC species, and residue part were all significant main effects (Table 2), and enzyme activity was, in general, lower in the low P than in the medium P soil (Figure 2B). In the medium P soil, the activities of phosphomonoesterase and phosphodiesterase, two enzymes directly related to the release of P from organic materials, were unaffected by CC residues and by additions of P fertilizer in reference treatments (Figure 2A,B, Table 3). In the low P soil, this was also true for the activity of phosphodiesterase, whereas phosphomonoesterase responded positively to treatments with lupine roots and roots + shoots in the low P soil (Figure 2A). These lupine residues accomplished significantly higher activities of phosphomonoesterase than corresponding residues of oats (Table 3). For lupine roots + shoots, this was also true for phosphodiesterase.

Compared with the no P reference, lupine roots + shoots residues increased the total microbial PLFA in the low P as well as the medium P soil (Figure 2C, Table 3); for medium P soil this was also true for lupine shoot residues. For these treatments, medium P soil was higher in microbial PFLA when exposed to lupine residues than to corncockle or oat residues. In the low P soil, lupine roots + shoots residues gave higher microbial PLFA compared with treatments with corncockle and oats residues. In general, species and type of CC residue affected total microbial PLFA significantly (Table 2), while soil P status had no impact. The bacteria-to-fungi ratio was unaffected by soil P status, CC species, and residue part. The arbuscular mycorrhizal fungi (AMF) abundance, estimated by the NLFA biomarker 16:1ω5, decreased when subject to the addition of P fertilizer in the reference treatments (Figure 2D), although this effect was significant only for the low P soil (Table 3). Overall, the AMF abundance was unaffected by CC residues although for treatments receiving corncockle roots + shoots, AMF abundance was higher in medium P soil than the low P soil. Residues of oats, the only mycorrhiza forming CC species in this experiment, had levels of the NLFA biomarker 16:1ω5 that were comparable to the reference treatment with no significant differences among CC species.

The CC species significantly affected the activity of β-glucosidase (Table 2). In the low P soil, the activity of this enzyme increased after the incorporation of lupine roots + shoots. In the medium P soil, lupine roots also resulted in a significantly higher activity of beta-glucosidase when compared with oats root residues. The type of CC residues significantly affected the activity of *N*-acetyl-glucosaminidase (Table 2). This arose from oats shoot residues in medium P soil providing a significantly higher activity than corncockle shoot residues (Table 4). In general, across all the microbial indicators, CC species and plant part had a larger impact than soil P status, while the performance of

TABLE 2 P values for main effects and interactions for all variables presented in figures and tables

	Main effects and interactions, *p*-values						
Variable	Soil P status	Species	Residues	Soil P status × Species	Soil P status × Species	Species × Residues	Soil P status × Species × Residues
Shoot dry matter	< **0.001**	< **0.001**	< **0.001**	**0.003**	< **0.001**	**0.002**	0.862
Phosphorus uptake	< **0.001**	**0.006**	< **0.001**	0.053	**0.003**	**0.006**	0.852
Number of tillers	< **0.001**	0.514	< **0.001**	**0.049**	0.196	0.228	0.528
P_{H2O}	< **0.001**	**0.018**	< **0.001**	0.179	0.485	0.152	0.933
Phosphomonoesterase	0.692	**0.002**	**0.009**	0.504	0.873	**0.003**	0.923
Phosphodiesterase	**0.029**	**0.045**	**0.081**	0.564	0.204	0.215	0.747
Total microbial phospholipid fatty acid	0.967	< **0.001**	**0.012**	0.866	0.711	0.285	0.421
Arbuscular mycorrhizal fungi abundance	0.191	0.420	0.233	0.200	0.706	0.056	0.713
Gram-positive bacteria	0.250	< **0.001**	**0.015**	0.975	0.329	0.617	0.307
Gram-negative bacteria	0.310	**0.013**	0.377	0.926	0.316	0.696	0.585
Fungi	0.484	0.382	0.922	0.495	0.965	**0.010**	0.951
Bacteria:fungi ratio	0.613	0.530	0.464	0.637	0.996	**0.021**	0.988
Beta-glucosidase	0.142	**0.029**	0.185	0.713	0.330	0.510	0.240
N-acetyl-glucosaminidase	0.340	0.508	**0.048**	0.981	0.621	0.265	0.053

Significant values $p < 0.05$ are in bold.

the barley test crop was little affected by soil P status, CC species, and residue part (Table 2).

4 | DISCUSSION

When incorporated in their vegetative stage, a very significant part of the total-P in CC residues is present as inorganic orthophosphate (Damon et al., 2014; Noack et al., 2012), a P pool that is potentially available for uptake in the crop that follows. However, following incorporation of CC residues, immobilization by microbes, and sorption by organic, and inorganic soil components reduce P availability, the sorption depending on soil P status (Johnston et al., 2014). This study focuses on the more persistent effects of CC incorporation on barley growth parameters and soil microbial properties, deliberately bypassing the initial short-term dynamics. Thus, we measured the effects of CC residues after an incubation period and growth of the main barley crop and not immediately after CC incorporation. This allows us to evaluate the longer lasting effects of CC growth and subsequent mineralization of CC residues on soil properties and growth of the main crop.

A basic concern associated with comparisons of soils with different P status relates to the different origins of low P and high P soils. When comparing soils from different sites, not only P status but also soil properties related to mineralogical composition and management history affect the impact of CC residues and the growth of test crops. Therefore, we based our study on soil from one well-controlled field experiment, with differences in soil P status established by long-term differences in fertilizer P additions. With this approach, we minimized confounding effects from soil properties other than soil P status and effects on, for example, the microbial community derived solely from differences in soil P status.

Neither for the low P nor the medium P soil, the addition of fertilizer P to reference soils without CC residues affected yields of barley biomass (Figure 1) whereas concentrations of water-extractable P (P_{H2O}) increased and more so in medium P than in low P soil. As both soils had similar barley yields, we assume similar C inputs to the soils and ascribe the smaller response solely to a larger P sorption capacity in the low P soil (Azeez et al., 2020).

The introduction of CC residues into soil-simulated harrowing/rotavating to 10 cm depth. For low P soil, the effect of CC residues on yield and P uptake of the barley test-crop, grown for 7 weeks before whole crop harvest, was generally small and insignificant compared with the no P reference soil (Table 3). The only exception was soil amended with lupine shoot material; for both soils, this treatment improved barley yields of DM and P (Figure 1). Reduced tillering can be a response to P deficiency (Carstensen et al., 2019; Rodríguez et al., 1999), and our results show fewer tillers in the low P soil than in medium P soil. The numbers of tillers were significantly higher in the corncockle shoots treatment than for oats, and lupine tended to be higher. This could indicate that more P had been available for barley in these two treatments at the time of tillering, supporting a higher yield.

Generally, the addition of CC residues had little effect on water-extractable P. For both soils, residues of corncockle roots + shoots provided a small but significant increase in P_{H2O} (Table 3). For all other treatments, CC residues did not affect P_{H2O} when compared to the unfertilized reference soils (no P). For DM yields of barley, P

TABLE 3 Statistical overview of the effects of cover crop residues illustrated in Figures 1 and 2

		Roots			Shoots			Roots + shoots		
Soil P status	**+ P fer.**	**Cornc.**	**Lupine**	**Oats**	**Cornc.**	**Lupine**	**Oats**	**Cornc.**	**Lupine**	**Oats**
Shoot dry matter yield										
Low	n/s	n/s	B	n/s	B	B*	A	B	B	n/s
Medium	n/s	*	A	n/s	Aa	Aa*	Bb	Ab	Aa	b
Phosphorus uptake										
Low	n/s	n/s	B	n/s	B	B*	B	B	B	*
Medium	n/s	*	A	n/s	Aa	Aa*	Ab	A	A	n/s
Water extractable P										
Low	*	B	B	B	B	B	B	Ba*	Bab	Bb
Medium	*	A	A	A	A	A	A	A*	A	A
Number of tillers										
Low	n/s	n/s	B	n/s	B	B	n/s	n/s	B	n/s
Medium	n/s	n/s	A	n/s	Aa	Aab	b	n/s	A	n/s
Phosphomonoesterase										
Low	n/s	b	a*	B	n/s	n/s	n/s	ab	a*	b
Medium	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s
Phosphodiesterase										
Low	n/s	n/s	n/s	n/s	n/s	n/s	n/s	a	ab	b
Medium	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/s
Total microbial phospholipid fatty acid										
Low	n/s	n/s	n/s	n/s	n/s	n/s	n/s	b	a*	B
Medium	n/s	n/s	n/s	n/s	b	a*	b	b	a*	B
Arbuscular mycorrhizal fungi abundance										
Low	*	n/s	n/s	n/s	n/s	n/s	n/s	B	n/s	n/s
Medium	n/s	n/s	n/s	n/s	n/s	n/s	n/s	A	n/s	n/s

*indicate significant difference from the reference treatment without P fertilization (no P) (Dunnett, $p < 0.05$). Lower case letters indicate significant differences between species within a residue treatment, and upper-case letters indicate differences between low P and medium P soil for a given species and residue treatment ($p < 0.05$). Cornc. , corncockle; fer., fertilizer; n/s, nonsignificant.

uptake, and water-extractable P, however, differences between the two soils were consistent with significantly higher values for medium P soil, except for treatments with oats residues. In accordance with this, Espinosa et al. (2017), using soils from a long-term field experiment with different annual inputs of mineral P fertilizer, concluded that the short-term transfer of P from CC to the following wheat crop was small and little affected by soil P status.

In conventional cereal cropping systems, incorporation of CC encompasses above- as well as belowground residues. However, most previous studies of effects of CC on specific soil properties and growth of subsequent crops consider aboveground residues only. Less is known regarding the specific effects of the shoot and root residues. J. Liu et al. (2013) studied the release of water-extractable P from shoots and roots of CC grown for 1 to 2 months and exposed to freeze–thaw treatments. On average, shoots contained 43% more water extractable P than roots, and P release related closely to total-P contents. Using litterbags incorporated under field conditions with the shoot and root biomass of CC, grown for 6 months, Talgre et al. (2014) observed that shoot residues released P faster than root residues during the following 6 months after incorporation. Our results align with these studies, as the overall effect of shoot residues on barley appeared to be neutral or positive, while the effect of root residues tended to be negative for the medium P soil and neutral for the low P soil with low yield levels.

Murrell et al. (2020) report a positive effect of CC associated with AMF on subsequent P content in maize seedlings. However, the effect of AMF root colonization on seedling P decreased as soil P content increased. We expected higher AMF abundance after harvest of barley in soil with oats roots than in soil amended with other CC residues because oats were the only AMF hosting CC included in this study. However, the abundance of AMF in soil remained unaffected by CC residues; the only significant effect was a decrease in AMF in the low P soil without residues but amended with mineral P fertilizer. It is well known that the addition of mineral P fertilizer reduces AMF abundance (e.g., Williams et al., 2017).

Several studies report a short-term increase in microbial activity after incorporation of CC arising from the input of easily available

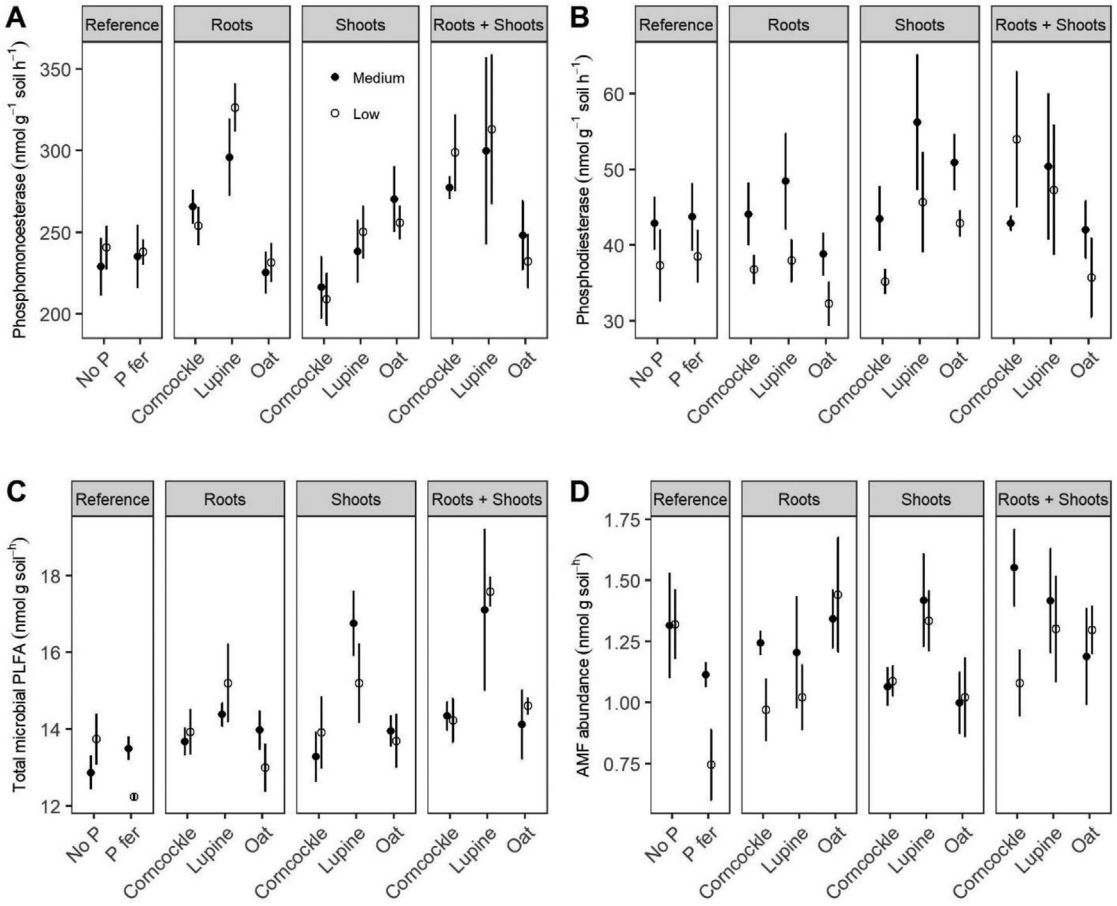


FIGURE 2 Activity of the enzymes phosphomonoesterase (A) and phosphodiesterase (B), total microbial PLFA (C), and AMF abundance (D) for treatments with cover crop residues and reference treatments. Filled circles show results from the medium P soil and open circles show results from the low P soil. Points are means, and error bars indicate standard errors ($n = 4$)

residue C (Bünemann et al., 2012; Hallama et al., 2019; Kim et al., 2020). The increased activity may induce immobilization of plant-available P originating from the soil and/or from inorganic P present in residues. Since this study attempted to establish any longer-lasting effects on the soil microbial community, soil sampling followed the whole-crop harvest of the barley, approximately 2 months after the incorporation of CC residues. Generally, the longer-term effects on soil microbial properties were small (Figure 2, Tables 3 and 4), the few significant effects were mainly linked to the positive effects of lupine residues. Thus, the treatments with lupine root + shoot residues had a positive impact on total microbial PLFA in both soils and lupine shoots also raised microbial PLFA in the medium P soil. Residues of lupine may represent an ideal substrate for microorganisms, an effect that appears to last during the growth of the following main crop. Furthermore, the fact that total microbial PLFA and the activity of phosphomonoesterase profits from incorporated lupine roots suggests that lupine roots offer an additional P and N resource. We recognize that the biomass of the N_2-fixing lupine provided a larger input of N than the two other CC species (Table 1). However, treatments with lupine residues did not reflect any consistent effect of the higher N input on microbial abundance and activity parameters.

The contribution of phosphodiesterase to the overall release of P from organic residues was much smaller than that from phosphomonoesterase, but it is noteworthy that soil P status affected phosphodiesterase activity but not phosphomonoesterase activity. The difference between soils may relate to a higher abundance of diesters in the medium P soil, for example, from nucleic acids in the microbial biomass, but this was not corroborated by higher PFLA biomarkers in the medium P soil. Certainly, the complex interactions and temporal dynamics of sorption, uptake, immobilization, and competition of organic P substrates among microbes and plants deserve further detailed scrutiny (George et al., 2018).

5 | CONCLUSIONS

We conclude that the longer-lasting effects of cover crop residues on the yield and P uptake in the following main crop depended on soil P status, CC species, and the quality of the CC residues. Lupine was the CC species giving the largest positive responses in the main crop. The responses in barley growth were generally positive or neutral to shoot material irrespective of soil P status, whereas for root material it was

TABLE 4 Amount of Gram-positive bacteria, Gram-negative bacteria, and fungi (nmol g^{-1}), and activity of the enzymes beta-glucosidase and *N*-acetyl-glucosaminidase (nmol g^{-1} soil h^{-1}). Raw means and standard errors in *italics* ($n = 4$)

Soil P status	Reference				Roots						Shoots						Roots + Shoots					
	No P		P fer		Corncockle		Lupine		Oats		Corncockle		Lupine		Oats		Corncockle		Lupine		Oats	
Gram-positive bacteria (nmol g^{-1})																						
Low	7.0	*0.3*	5.9	*0.2**	6.5	*0.4ab*	7.2	*0.5a*	6.2	*0.5b*	6.6	*0.4*	7.2	*0.4*	6.9	*0.6*	6.8	*0.2b*	8	*0.7a*	7.1	*0.4b*
Medium	6.4	*0.3*	6.5	*0.2*	6.9	*0.4*	7.3	*0.4**	6.9	*0.4*	6.4	*0.3b*	7.7	*0.3** *a*	6.8	*0.2b*	7.1	*0.4ab*	7.8	*0.3** *a*	6.9	*0.4b*
Gram-negative bacteria (nmol g^{-1})																						
Low	2.3	*0.6*	2.4	*0.4*	2.7	*0.5*	3.4	*0.5A*	2.2	*0.5*	2.4	*0.6*	2.7	*0.6*	2.5	*0.4*	2.6	*0.5*	3.1	*0.8*	2.8	*0.5*
Medium	2.4	*0.5*	2.5	*0.4*	2.2	*0.5*	2.3	*0.4B*	2.1	*0.5*	2.2	*0.5*	3	*0.7*	2.7	*0.4*	2.4	*0.6*	3.4	*0.8*	2.4	*0.6*
Fungi (nmol g^{-1})																						
Low	1.03	*0.26*	0.98	*0.24*	1.24	*0.39*	0.81	*0.16*	1.08	*0.21*	1.36	*0.33*	1.03	*0.09*	0.74	*0.10*	0.82	*0.08*	1.48	*0.45*	0.75	*0.12*
Medium	0.83	*0.22*	1.20	*0.44*	0.85	*0.15*	0.76	*0.12*	1.20	*0.35*	1.15	*0.35*	1.07	*0.19*	0.79	*0.10*	0.73	*0.09*	1.28	*0.31*	0.80	*0.16*
Bacteria:fungi ratio																						
Low	11.5	*3.1*	10.3	*2.7*	9.6	*2.4*	14.3	*4.2*	8.7	*1.7*	7.7	*1.5*	10.1	*1.2*	13.6	*2.5*	11.9	*1.2*	9.8	*3.8*	14.1	*2.1*
Medium	12.5	*2.5*	10.4	*2.9*	11.6	*1.7*	13.7	*2.4*	9.1	*2.0*	9.2	*2.1*	11.0	*2.1*	12.4	*1.4*	13.4	*1.3*	10.5	*2.3*	13.2	*2.7*
Beta-glucosidase activity (nmol g^{-1} soil h^{-1})																						
Low	120	*15*	143	*8*	135	*11*	132	*9B*	134	*6*	137	*4*	155	*15*	138	*10*	158	*9*	179	*28**	149	*16*
Medium	144	*4*	129	*10*	145	*10ab*	189	*36Aa*	132	*18b*	139	*9*	158	*8*	174	*15*	157	*17*	177	*23*	142	*15*
N-acetyl-glucosaminidase activity (nmol g^{-1} soil h^{-1})																						
Low	41	*3*	36	*2*	37	*1*	42	*2*	42	*8*	41	*3*	46	*6*	39	*2B*	47	*1*	44	*1*	42	*4*
Medium	40	*4*	40	*2*	44	*4*	40	*4*	35	*2*	36	*3b*	44	*5ab*	54	*4Aa*	50	*2*	53	*9*	43	*6*

*indicate significant difference from the reference treatment without P fertilization (no P) (Dunnett, $p < 0.05$). Lower case letters indicate significant differences between species within a given residue treatment, and upper case letters indicate differences between low and medium P soil for a given species and residue treatment ($p < 0.05$)

negative on medium P soil and neutral on low P soil. Only lupine roots and shoots provided lasting effects on the soil microbial structure and activity, detectable after the growth of the main crop.

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent assistance provided by the technical staff at Askov Experimental Station and Department of Agroecology, Aarhus University, and at Soil Biology Department, University of Hohenheim. Moritz Hallama, Ellen Kandeler, and Gitte Rubæk received funding from the *European Union's Horizon 2020 research and innovation programme* under *grant agreement No 456 677407* (*SoilCare project*).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Haward dataverse at https://doi.org/10.7910/DVN/4GMJKV.

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How to cite this article: Christensen, J. T., Hansen, E. M., Kandeler, E., Hallama, M., Christensen, B. T., & Rubæk, G. H. (2021). Effect of soil P status on barley growth, P uptake, and soil microbial properties after incorporation of cover crop shoot and root residues. *Journal of Plant Nutrition and Soil Science*, *184*, 657–667. https://doi.org/10.1002/jpln.202100046