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ORIGINAL ARTICLE

Influence of cover crop residue traits on phosphorus availability and subsequent uptake by plants

Nicolas Honvault · Michel-Pierre Faucon · Timothy McLaren · David Houben · Emmanuel Frossard · Astrid Oberson

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Abstract Cover crops are typically thought to increase the P nutrition of crops. However, there are mixed reports on this with some studies reporting a negative effect. An improved understanding of cover crop residues and their P release dynamics could offer new insight with the benefit of improved management for optimal P availability in cropping systems. Here, we examined the influence of cover crop residue traits for six different crop types on soil P availability and subsequent plant (ryegrass) P uptake over a four-month period in a soil with moderate P availability. Among the residue traits examined (residue P concentration, N concentration, C:P ratio, C:N ratio, N:P ratio and specific leaf area), only residue P concentration and C:P ratio were related to soil P availability and subsequent crop P uptake. Important short-term effects of residue C:P ratio on P availability were highlighted. Strong to moderate negative correlations between residue C:P ratio and subsequent crop P uptake (R^2 between 0.4 and 0.8) were observed. Decreases in subsequent crop uptake of up to 43% compared to unfertilized pots occurred for residues with high C:P ratios for the first cut, strongly suggesting microbial P immobilization. Effects faded with time, with most cover crop residue additions having little to no influence on ryegrass P uptake over a four month period. Residues with the highest C:P ratio nonetheless resulted in a 22% decrease in cumulative P uptake compared to unfertilized pots. Our study highlighted that cover crop C:P ratio should be managed in order to ensure minimized adverse effects of microbial P immobilization. The observed low effects of cover crop residues on P uptake in a subsequent crop suggest that improving P availability in context with moderate P limitations via cover cropping may require relying on other services provided by cover crops such as mobilization of sparingly available P pools.

Keywords Cover crop · Phosphorus · C:P ratio · Functional ecology

Abbreviations

0P	Control treatment without phosphorus addition
BraC	*Brassica carinata*
FagE	*Fagopyrum esculentum*

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N. Honvault (✉) · M.-P. Faucon · D. Houben
AGHYLE UP 2018.C101, UniLaSalle, 60000 Beauvais, France
e-mail: nicolas.honvault@cegetel.net

T. McLaren · E. Frossard · A. Oberson
Group of Plant Nutrition, Institute of Plant Sciences, Swiss Federal Institute of Technology (ETH), P.O. 185, Lindau, Switzerland

T. McLaren
School of Agriculture and Food Sustainability, The University of Queensland, St Lucia, QLD 4072, Australia

LenC *Lens culinaris*
minP Treatment with addition of 15 mg P kg^{-1} soil mineral fertilizer (KH_2PO_4)
PhaC *Phacelia tanacetifolia*
P_{mic} Soil microbial P content based on difference method between fumigated and unfumigated resin extracts
P_{res} Soil available P content as extracted by anion exchange resins
SLA Specific leaf area
VicF *Vicia faba*
VicV *Vicia villosa*

Introduction

Low bioavailability of soil phosphorus (P) in most agroecosystems often requires the addition of P fertilizer. However, a large portion of the added P is sorbed onto soil mineral surfaces, reducing plant P uptake and resulting in low P fertilizer use efficiencies (Richardson et al. 2011). Over time, the regular addition of P fertilizer results in the accumulation of sparingly-soluble forms of soil P (Bouwman et al. 2017). Improving crop utilisation of sparingly-soluble forms of soil P could lead to increased P cycling efficiency and reduced dependency on declining resources of phosphate rocks (Faucon et al. 2015; Richardson et al. 2011; Simpson et al. 2011). Several strategies are used by plants to mobilize pools of sparingly-soluble soil P (Richardson et al. 2011). Selection for targeted traits in crops or selection of suitable species in multispecies systems such as cover crops, could lead to improved P cycling efficiency in agroecosystems (Faucon et al. 2015; Honvault et al. 2021).

Cover crops can be selected to offer many ecosystem services, such as carbon (C) sequestration, enhanced nutrient cycling, or atmospheric nitrogen (N) fixation (Daryanto et al. 2018). Lately cover crops have also been increasingly suggested to play an important role in enhancing or maintaining P availability (Damon et al. 2014; Hallama et al. 2019; Hansen et al. 2021). Some studies have reported increases of up to 50% in crop P uptake for the subsequent crop after cover-cropping when compared to bare soil (Hallama et al. 2019). However, the cover-cropping effects on P uptake by the subsequent crop is highly variable across pedoclimatic conditions, with several studies reporting neutral to negative effects (Hallama et al. 2019). This might be explained by the wide range of traits among cover crop species (Alamgir et al. 2012; Hansen et al. 2022; Maltais-Landry and Frossard 2015; Noack et al. 2012). In addition, cover crop effects on P availability are also modulated by initial soil P availability, possibly contributing to this wide range of effects (Damon et al. 2014; Hallama et al. 2019; Hansen et al. 2022).

Cover crops can acquire P from soil P pools of varied spatial and chemical availability, based on differences in their architectural, physiological and morphological traits (Richardson et al. 2011). Root morphological traits may improve P foraging, supporting higher crop yield and P uptake at low concentrations of soil P fertility (Haling et al. 2018; Ma et al. 2018; Pang et al. 2010). Release of exudates such as carboxylates or acidifying agents can mobilize sparingly-soluble soil P by decreasing P sorption on mineral surfaces or solubilizing calcium phosphate in calcareous soils (Li et al. 2017; Wang and Lambers 2020; White and Hammond 2008). Exudation of enzymes may also improve the mineralization of slowly mineralizing organic P pools (Nobile et al. 2019; Richardson et al. 2011). Through these traits cover crops can substantially increase soil P availability (Hansen et al. 2021), but also accumulate significant amounts of P in their biomass from the diverse soil P pools (e.g., 5–30 kg P ha^{-1}) (Damon et al. 2014; Maltais-Landry and Frossard 2015; Ruis et al. 2019; Wendling et al. 2016). During decomposition of the unharvested cover crop biomass left on the field, P acquired by cover crops is then released, potentially resulting in improved P nutrition in subsequent crops (Dube et al. 2017). However, the contribution of P release from cover crop residues to soil P availability has been proposed to be agronomically significant only in conditions where large amounts of P is acquired and later released by cover crops (Damon et al. 2014; Thibaud et al. 1988). Large variability in the potential contributions of cover crop residues to P availability underline the need to further investigate the factors involved in the fate of residue P in order to optimize cover crops composition and management for enhanced benefits on soil P availability (Faucon et al. 2015).

Phosphorus release from crop residues during their decomposition is a complex process generally involving a pattern of rapid initial P leaching of inorganic P from residues to the soil solution followed by a

slower release of P (Prescott 2005). Upon release, P can either remain plant available, be adsorbed onto soil particles, or become immobilized by soil microorganisms (Alamgir et al. 2012; Damon et al. 2014; Prescott 2005; Simpson et al. 2011). If the amount of soluble P does not meet microbial requirements, residue P can be immobilized into microbial biomass, to be later released during microbial turnover typically within a few days to a month after residue addition (Damon et al. 2014; Spohn and Widdig 2017).

Many factors influence P release from residues among which residue C, N but especially P concentration. In general, a rapid release of P from residues can be expected for concentrations higher than 2.5 to 3 g P kg^{-1}, while concentrations below 2.4 g P kg^{-1} are expected to result in a slower P release or even P immobilization (Kwabiah et al. 2003; Alamgir et al. 2012; Damon et al. 2014). However, much uncertainty remains on these general threshold values (Kwabiah et al. 2003). While less investigated, there is increasing evidence that residue architecture reflected in part by traits such as specific leaf area (SLA) also play a central role in residue decomposition and nutrient release (Freschet et al. 2013; Perez-Harguindeguy et al. 2013; Zukswert and Prescott 2017). Tissue architecture may be important in residue degradation and later nutrient release due to its role in early leaching dynamics and later decomposer access (Lindedam et al. 2009; Zukswert and Prescott 2017). Specific leaf area has for instance been observed to strongly relate to residue decomposition rates across a range of natural ecosystems (Liu et al. 2018; Santiago 2007). Lower SLA may slow residue decomposition and nutrient release due to reduced surface area per mass slowing early leaching dynamics and reducing decomposer access (Lindedam et al. 2009; Zukswert and Prescott 2017). Reduced surface area for larger residue pieces has for instance been proposed to explain a slower release of residue P (Noack et al. 2014).

Stochiometric ratios between residue C, N and P have also been highlighted to play an important role in residue decomposition and nutrient release. Indeed soil microorganisms tend to be primarily C-limited, and co-limited by elements necessary to decompose C-sources (Smith and Paul 1990). Negative correlations have been observed between nutrient ratio such as C:N or C:P and residue decomposition due to microbial N and P requirements to decompose C-sources (de la Riva et al. 2019; Freschet et al. 2013; Lin et al. 2020). However, much uncertainty remains as to how much P is taken up by microbial biomass during residue decomposition with values ranging from 1.5 to 3 mg P g^{-1} DM (Kwabiah et al. 2003). Residues with C:P ratios higher than 300 were reported to lead to microbial P immobilization, while this threshold value tends to vary greatly with reports of values between 60 and 700 (Espinosa et al. 2017). Residues' C:P ratio effects on P availability via microbial P immobilization in turn can be inconsistent and potentially misleading according to the various organic P compounds in residues (Enwezor 1976; Umrit and Friesen 1994; White and Ayoub 1983).

Key factors influencing P release from residues and their effect on P availability for the subsequent crop have yet to be fully investigated for cover crop residues (Espinosa et al. 2017; Hansen et al. 2022). Most studies focused on residue from mature crops (Alamgir et al. 2012; Noack et al. 2014), possibly underestimating the effects of vegetative stage residues such as cover crop residues (Maltais-Landry and Frossard 2015). Alongside the different range of traits of young rather than mature residues, morphological traits influence has received little attention ignored due to the use of milled residues (Damon et al. 2014; Noack et al. 2014). Furthermore, relationships between leaf traits and nutrient dynamics identified in ecosystems (de la Riva et al. 2019) are rarely investigated due to limitations using whole-plant milled residues. Leaves nonetheless often present higher nutrient concentrations and constitute a large part of residues. Effects of leaf traits may be partly masked when leaves get mixed with stems. The large range of contribution from residue to the P taken up by subsequent crop, from 1 to 45% (Nachimuthu et al. 2009; Noack et al. 2012; Thibaud et al. 1988) reported prompts further investigation in the role of cover crop traits in driving P release dynamics from residues and their contribution to P uptake by the subsequent crop. Getting a better understanding of key cover crop traits involved in these dynamics could offer to manage and select cover crops for enhanced P cycling efficiency in agrosystems (Espinosa et al. 2017; Faucon et al. 2015).

We investigated the influence of cover crop residues traits on the fate of residue P in the plant-soil-microbe system during a greenhouse and an incubation experiment. The effects on P uptake by the

following crop (Italian ryegrass, *Lolium multiflorum* L.) were investigated in a greenhouse experiment with six cover crop species selected for their gradient of traits. The effects on soil P pools (resin extractable and microbial P pools) were investigated with an incubation experiment. We tested the hypotheses that i) residues with high P concentration or high SLA will release more P faster, contributing more to soil P availability and subsequent plant uptake than residues with lower P concentration or SLA, ii) residues with higher C:P ratio will result in delayed or reduced contribution to soil P availability and subsequent plant uptake compared to residues with lower C:P ratio.

Material and methods

Soil collection and preparation

A Retisol soil (formerly called Albeluvisol) (IUSS Working Group 2006) at a depth of 5 to 20 cm was collected from a cultivated field in north-eastern France (48° 54′ 37″ N, 3° 43′ 57″ E). The surface layer (0–5 cm) was removed prior to collection in order to obtain soil with lower concentrations of 'plant-available' P. The soil was dried at ambient temperature for 60 days and then passed through a 2 mm sieve. Dried soil was stored in the dark for about a year before use in the experiment. The concentration of Olsen-P in the collected soil was 16.3 mg Olsen-P kg^{-1}, which is considered moderately P limited (Hansen et al. 2022) (Table 1). Soil organic C was 12.8 g C kg^{-1} and pH_{KCl} was 7.4.

Table 1 Chemical and physical properties of the soil used in the study

Soil properties	
Clay (g kg^{-1})	256
Silt (g kg^{-1})	676
Sand (g kg^{-1})	68
Total N[a] (g kg^{-1})	1.42
Organic C[b] (g kg^{-1})	12.8
C/N	9
CEC (cmolc kg^{-1})	10.5
Exchangeable Ca[c] (g kg^{-1})	3.30
Exchangeable K[c] (g kg^{-1})	0.28
Exchangeable Mg[c] (g kg^{-1})	0.14
Olsen P[d] (mg kg^{-1})	16.3
HCl P[e] (mg kg^{--1})	76.7
Total P[f] (mg kg^{-1})	600

[a]Dumas method (NF ISO 13 878, AFNOR 1998)

[b]Sulfochromic oxidation (NF ISO 14235, AFNOR 1998)

[c]Exchangeable, Extracted with ammonium acetate 0.5 M, EDTA 0.02 M pH 4.65 (NFX 31-108, AFNOR 2002)

[d]According toOlsen (1954)

[e]HCl P as extracted with 1 M HCl from solid residue after initial extraction with NaOH 1 M as defined inGarcía-Albacete et al. (2012)

[f]ICP-AES after total solubilization with hydrofluoric and perchloric acid (NF X 31-147, AFNOR 1996)

Preparation of crop residues

Residues from six cover crop species were selected for their diverse phylogenetic lineages and traits (Brassicaceae: *Brassica carinata* (BraC) A. Braun; Polygonaceae: *Fagopyrum esculentum* (FagE) Moench.; Fabaceae: *Lens culinaris* (LenC) Medik., *Vicia faba* (VicF) L., *Vicia villosa* (VicV) Roth.; Hydrophyllaceae: *Phacelia tanacetifolia* (PhaC) Benth.) (Table 2). Cover crop residues were obtained from crops grown in the glasshouse on the aforementioned Retisol soil diluted with washed sand (22% mass) to further decrease concentrations of plant-available soil P. Cover crops were grown in multiple periods of approximately 2.5 months each in a replicated random design. At harvest, plants were mostly toward the end of their vegetative stage with the first onset of flowering visible. Growth conditions were set at a photoperiod of 14 h day^{-1}, with 22 °C at day and 18 °C at night. Plants were watered twice a week to maintain soil humidity approx. between 60 and 50% of soil water holding capacity. The biomass of each batch was combined and homogenized. After homogenisation, the dried cover crop residues were divided into two portions. Leaves for the 'leaf residues' treatment were collected in one portion. The other portion, consisting of both leaves and stems was used in the 'aboveground residues' treatment. Leaf specific area of residues was determined via scanning leaves issued from the homogenised biomass pool before separation using an Epson Scanner perfection V800 to produce a 600 dpi image. The image was then analysed using imageJ software (version 1.53) to determine leaf area. After 48 h drying at 55 °C, scanned leaves were weighed to calculate their SLA (in mm^2 mg^{-1}). The residues were then manually cut into pieces to

Table 2 Cover crop residue traits and inputs for each residue treatment (all inputs designed to add 15 mg P kg^{-1} soil)

Residues	Species	P g kg^{-1} DM	N	C	C:N	C:P	N:P	SLA mm^2 mg^{-1}	N added mg kg^{-1} soil	C added mg kg^{-1} soil
Aboveground	BraC	2.44	18.6	400	21.5	164	7.6	21.7	114	2456
	FagE	2.49	9.2	403	43.9	162	3.7	47.4	55	2434
	LenC	2.26	23.0	429	18.7	189	10.1	31.3	152	2842
	PhaC	2.73	8.6	379	44.0	139	3.2	24.2	47	2083
	VicF	1.85	26.3	415	15.8	224	14.2	38.5	213	3362
	VicV	1.28	22.5	404	18.0	316	17.6	38.8	264	4739
Leaf	BraC	1.86	25.2	404	16.0	217	13.6	21.7	203	3257
	FagE	2.41	24.4	377	15.5	156	10.1	47.4	152	2347
	LenC	2.71	35.1	423	12.0	156	13.0	31.3	194	2340
	PhaC	2.54	18.4	352	19.2	139	7.2	24.2	109	2082
	VicF	3.07	49.9	422	8.5	137	16.2	38.5	244	2060
	VicV	2.04	35.1	414	11.8	204	17.2	38.8	259	3054

SLA Specific leaf aera. BraC—*Brassica carinata*; FagE—*Fagopyrum esculentum*; LenC—*Lens culinaris*; PhaC—*Phacelia tanacetifolia*; VicF—*Vicia faba*; VicV—*Vicia villosa*

replicate mechanical shredding in the field. In order to conserve mass to area ratio of cover crops (SLA) and examine its effects, residues were manually cut into pieces of 25 mm^2. This was done to examine the effect of morphology on the fate of residue P in the plant-soil system.

Experimental design

To investigate the influence of residue traits, namely residue P, N and C concentration, C:P ratio, C:N ratio, N:P ratio and SLA, on the fate of residue P in the plant-soil-microbe system, a glasshouse and an incubation experiment were established. Experimental treatments included the addition of two types of plant residues to the soil (i.e., the residues of (i) leaves and that of (ii) aboveground biomass) from six cover crop species, the addition of mineral P fertilizer (minP), and an unfertilized control (0P). Aboveground residues consisting of leaves and stems were investigated as most representative of field cover crops residues. Leaf residues alone were also examined as leaves are known as one of the main drivers of nutrients dynamic in ecosystems. However, the effects of leaf traits (SLA, P, C:P) may be partly masked when leaves get mixed with stems. Relationships between residue traits and the fate of residue P in the plant-soil-microbe system were examined separately per biomass type (i.e., leaf residues or aboveground residues) as well as for all residues regardless of biomass type. All plant residues and the mineral fertilizer were added to supply 15 mg P kg^{-1} soil. This rate corresponds to a field productivity of 6.5 t DM ha^{-1} for residues with a P concentration of 3 mg P kg^{-1} (assuming 1300 t soil ha^{-1}), and is similar to that reported in previous studies (Maltais-Landry and Frossard 2015; Noack et al. 2014). The experiment was arranged in a factorial randomized complete design and replicated four times.

Pot experiment set up

The aforementioned soil was rewetted at gravimetric water content (GWC) of 156 g H_2O kg^{-1} soil, which is equivalent to 41% of the soil's maximum water holding capacity (WHC), in order to increase microbial activity. After 10 days of incubation, the soil for the glasshouse and incubation experiment was labelled with a carrier free ^{33}P H_3PO_4 radiotracer at 2.1 MBq kg^{-1} soil. Briefly, 1 kg of dry-weight equivalent soil was weighed and a 10 mL aliquot of 216 MBq L^{-1} carrier free ^{33}P H_3PO_4 solution was added evenly across the soil. The soil was then mixed for 2 min, adjusted to 60% of its WHC, and then left to equilibrate.

After 18 days the P treatments were added to the soil and mixed for 2 min. This included the aforementioned crop residues or 10 mL of a solution of 1.5 g P L^{-1} mineral fertilizer as KH_2PO_4. The unfertilized control was also mixed for 2 min with 10 mL distilled

water, as for the fertilized treatments. A direct labelling control treatment was also included, adding 10 mL of a solution containing 1.5 g P L^{-1} mineral fertilizer and 205 MBq L^{-1} carrier free ^{33}P H_3PO_4 (Approx. SA 137 kBq mg^{-1} P). Lastly, all soils simultaneously received basal nutrients except for P at the following rates (mg kg^{-1} soil): 120 N, 250 K, 40 Ca, 50 Mg, 1 Fe, 150 Cl, 1 B, 2 Mn, 1 Zn, 2 Cu, 1 Mo (as NH_4NO_3, K_2SO_4, $MgSO_4$, $CaCl_2$, $MnSO_4$, $ZnSO_4$, $CuSO_4$, Na_2MoO_4, H_3BO_3 and $C_{10}H_{12}FeN_2NaO_8$).

Italian ryegrass (*Lolium multiflorum* L.) seeds (0.5 g) were added to each pot and placed 10 mm below the soil surface. This corresponded to an input of 1.6 mg P kg^{-1} soil, which is approximately 10% of the P contained in the added crop residues. Soils were kept between 50 and 60% of their WHC (during ryegrass growth) with distilled water. Growth conditions were set at a photoperiod of 14 h day^{-1}, with 24 °C at day and 18 °C at night. Pots were randomized weekly.

Ryegrass shoots were harvested five times 2 cm above the soil surface (day 35, 47, 70, 91 and 110 after the treatment application). After each harvest, the plants were supplied with all nutrients except for P at the following rates (mg kg^{-1} soil): 120 N, 250 K, 40 Ca, 50 Mg, 1 Fe, 150 Cl, 1 B, 2 Mn, 1 Zn, 2 Cu, 1 Mo (as NH_4NO_3, K_2SO_4, $MgSO_4$, $CaCl_2$, $MnSO_4$, $ZnSO_4$, $CuSO_4$, Na_2MoO_4, H_3BO_3 and $C_{10}H_{12}FeN_2NaO_8$). Nitrogen was added in two equal doses of 60 mg kg^{-1} one week apart.

Plant analyses and calculations

All ryegrass shoots were dried at 45 °C for 72 h, weighed, and then ground to powder using an MM 300 Mixer Mill (Qiagen, USA). Concentrations of total P in shoot material were determined using the method of Hoenig (2001). Briefly, 0.2 g of shoot material was digested with 2 mL HNO_3 69% (v/v) for 1 h at 200 °C with a MLS Turbowave (MWS GmbH, Heerbrugg, Switzerland) and then diluted in 10 mL of distilled water. Concentrations of P in digests were then determined via colorimetry using malachite green (Ohno and Zibilske 1991). Concentrations of total C and N in shoot material were determined via dry combustion.

The ^{33}P activity in plant material was determined via scintillation counting after mixing 2 mL of the plant digests with 5 mL of scintillation liquid (Ultima Gold AB, Packard Instrument Co.). The specific activity (SA) was then calculated based on measured P concentration in biomass and dry biomass weight. Specific activity in the first two cuts was corrected for 60% seed P uptake in the first cut and 20% seed P uptake in the second cut (Hansen et al. 2022; Nanzer et al. 2014; Noack et al. 2014) as follows:

$$SA_{Ryegrass} = \frac{Plant\ Activity\ (Bq)}{Plant\ P(mgP) - Correction\ factor * Seed\ P(mgP)}$$

$SA_{Ryegrass}$: Corrected specific activity in ryegrass biomass, Plant activity: Activity in ryegrass digest (Bq), Plant P: Ryegrass P uptake (mg P), Correction factor: Correction factor for seed P uptake, 60% for the first cut and 20% for the second, Seed P: Total seed P content (mg P).

P derived from fertilizer (Pdff) was calculated for the direct labelling control, adding 10 mL of a solution of 1.5 g P L^{-1} mineral fertilizer containing 205 MBq L^{-1} carrier free ^{33}P H_3PO_4, was calculated as follow:

$$Pdff = SA_{Plant}/SA_{Fertilizer} \times Plant\ Puptake(mgP)$$

Pdff: P derived from fertilizer (mg P), SA_{Plant}: SA of P in plants grown with ^{33}P labeled mineral fertilizer (kBq mg^{-1} P), $SA_{Fertilizer}$: SA of applied labeled mineral fertilizer (kBq mg^{-1} P).

To assess the nutritional status of plants, the nitrogen nutrition index (NNI) and P nutrition index (PNI) of shoots were calculated according to the methods described in Lemaire et al. (2008) and Duru and Ducrocq (1996). Nitrogen nutrition index was calculated as follows:

$$NNI = \frac{Na}{Nc} = \frac{Na}{4.8 \times DM^{-0.32}}$$

With Na: N content (g 100 g^{-1}), Nc: Critical N content, as calculated as function of dry matter (g 100 g^{-1}), DM: Plant dry biomass (t ha^{-1}).

And PNI as follows:

$$PNI = \frac{Pa}{Pc} = \frac{Pa}{0.15 + 0.065 \times Na}$$

With Pa: P content (g 100 g^{-1}), Pc: Critical P content, as calculated as a function of N content (g 100 g^{-1}), Na: N content (g 100 g^{-1}).

For NNI calculation ryegrass biomass per pot (1 kg soil) was converted to t ha^{-1} with the same

assumptions as for P addition rate calculations ie 1300 t soil ha^{-1}.

Soil incubation design and set up

Soil in the incubation experiment was amended at the same rates as for the glasshouse experiment. Soil portions for the incubation were of 100 g dry soil. Soil was labelled with a carrier free ^{33}P H_3PO_4 radiotracer at 2.1 MBq kg^{-1} soil and mixed for 2 min. Soil then was adjusted to 60% of its WHC, and left to equilibrate for 18 days before amendment with P treatments. During soil amendment with the P treatments, the soil also received basal nutrients except for P at the following rates (mg kg^{-1} soil): 120 N, 250 K, 40 Ca, 50 Mg, 1 Fe, 150 Cl, 1 B, 2 Mn, 1 Zn, 2 Cu, 1 Mo (as NH_4NO_3, K_2SO_4, $MgSO_4$, $CaCl_2$, $MnSO_4$, $ZnSO_4$, $CuSO_4$, Na_2MoO_4, H_3BO_3 and $C_{10}H_{12}FeN_2NaO_8$). The soil was then mixed for 2 min before being kept in the dark under identical experimental conditions as for the previously mentioned pot experiment. Soil was then sampled 10 and 59 days after amendment. Any remaining visible residue pieces were separated from the soil using a pair of tweezers before resin P and microbial P measurement (as detailed in 2.3.4). A subsample of soil from every treatment was used for gravimetric water content determination.

Soil analyses

Microbial P (P_{mic}) was determined via the difference in P concentration between hexanol fumigated and non-fumigated soil extracts using anion exchange resins (Bünemann et al. 2007). After preparation with 0.5 M $NaHCO_3$, the anion exchange strips were shaken for 16 h with 2 g equivalent dry soil and 30 mL deionised water. Each incubated soil was extracted in triplicate, one (i) with or (ii) without the addition of 1 mL hexanol as a fumigant, and one (iii) that received a P spike of a known amount of inorganic P (15 mg P kg^{-1} as KH_2PO_4) to correct for P sorption. The anion-exchange strips (VWR, 551642S) were then rinsed with deionised water before having their P extracted by shaking 1 h in 30 mL 0.5 M HCl. For the second extraction after 59 days resins were eluted with 10 mL 0.5 M HCl instead to ensure measurable amount of radioactivity. To compensate for sorption of microbial P released after fumigation, P recovery (58% of the added 15 mg P kg^{-1} on average for soil amended with organic amendments) was calculated based on spiked samples otherwise extracted identically to non-fumigated samples. Extracted P in the three extracts was measured via malachite green colorimetry (Ohno and Zibilske 1991). Microbial P was calculated as the difference between fumigated and non-fumigated samples, and corrected for the P recovery of added P:

$$P\,mic = \frac{(P\,fum - Pres)}{P\,recovery}$$

With P mic: Microbial P (mg P kg^{-1}), P fum: P in fumigated extract (mg P kg^{-1}), P res: P in unfumigated resin extract (mg P kg^{-1}), P recovery: fraction of P recovered in spiked extracts.

Radioactivity was measured by scintillation counting after mixing 1 ml of extract with 5 ml of scintillation liquid (Ultima Gold AB, Packard Instrument Co.). Specific activity in microbial extracts was calculated based on SA difference between fumigated and resin extracts. Prior to calculation SA in the fumigated extract was corrected for P sorption with the same factor as for microbial P, assuming similar sorption between ^{31} and ^{33}P.

Statistical analysis

To examine the overall effects of treatments and time since amendment on ryegrass biomass and P uptake, linear mixed models with random effects at pot level due to repeated measurements were used. Models with treatment, time and their interactions were selected based on lowest Akaike information criterion (AIC) and normal residual distribution. Differences between treatments per time point (ie. 35, 47, 70, 91 or 110 days after addition for the greenhouse experiment and 10 and 59 days after addition for the incubation experiment) were examined with one-way analyses of variance (ANOVAs) and post hoc tests of Tukey or Kruskall-Wallis tests and post hoc test of Mann Whitney if ANOVA requirements were not met. Tests were performed separately per residue type (leaf or aboveground residues) as ANOVAs and Kruskall-Wallis tests showed significant effects of residue types.

To examine relationships between residue traits and ryegrass P uptake and soil P availability, correlations were examined via Pearson correlation tests (r)

on log transformed data or Spearman correlation tests (rs) for variables not normally distributed after log transformation. Correlations between residue traits and P uptake and availability were first examined separately per residue type. Correlations between the same residue traits and P uptake (or P availability) were identified for both residue types. Residue traits effects were then simultaneously examined on both residue types. This also allowed assessing the relationships over a wider trait-range. To further investigate the relationship between residue traits and P uptake, linear models were produced. Residue traits correlated with P uptake were tested as predictors of P uptake in ryegrass. Soil P pools were also tested as predictors of P uptake in ryegrass as well as combinations of residue traits and soil P pools. Significant models were compared based on adjusted R^2 with the highest relative value considered the best fit. All tests were performed in R version 3.6.0 and the packages Rcmdr (2.8–0, Fox and Bouchet-Valat 2022), multcomp (1.4–20, Hothorn et al. 2008), nmle (3.1–160, Pinheiro et al. 2020), ggplot2 (3.4.0, Wickham 2016), vegan (2.6–4, Oksanen et al. 2022) with a significance level of 0.05.

Results

Cover crop residue properties

Concentrations of P in cover crop residues ranged from 1.28 g P kg^{-1} for VicV aboveground residues to 3.07 g P kg^{-1} for VicF leaf residues (Table 2). The C:P ratios of the cover crop residues ranged from 137 for VicF leaf residues to 316 for VicV aboveground residues, which corresponded to the addition of 2.1 g C kg^{-1} to 4.7 g C kg^{-1}. Aboveground residues of FagE resulted in the lowest N addition rate of 47 mg N kg^{-1} soil and VicV aboveground residues the highest of 264 mg N kg^{-1}. BraC had the lowest observed SLA of 21.7 mm^2 mg^{-1}, while the highest SLA of 38.8 mm^2 mg^{-1} was observed for VicV.

Effect of cover crop residues on ryegrass growth, biomass and nutrition

Cumulative ryegrass biomasses were largely similar across all treatments at the end of the experiment (Fig. 1). Average ryegrass biomass per cut decreased with time, from 4.4 g kg^{-1} soil after 35 days to 3.5 g kg^{-1} soil after 110 days. Significantly lower cumulative ryegrass biomass was only observed in pots amended with VicV aboveground residues as compared to VicF aboveground residues (19.9 g kg^{-1} soil compared to 21.7 g kg^{-1} soil) (Fig. 1a).

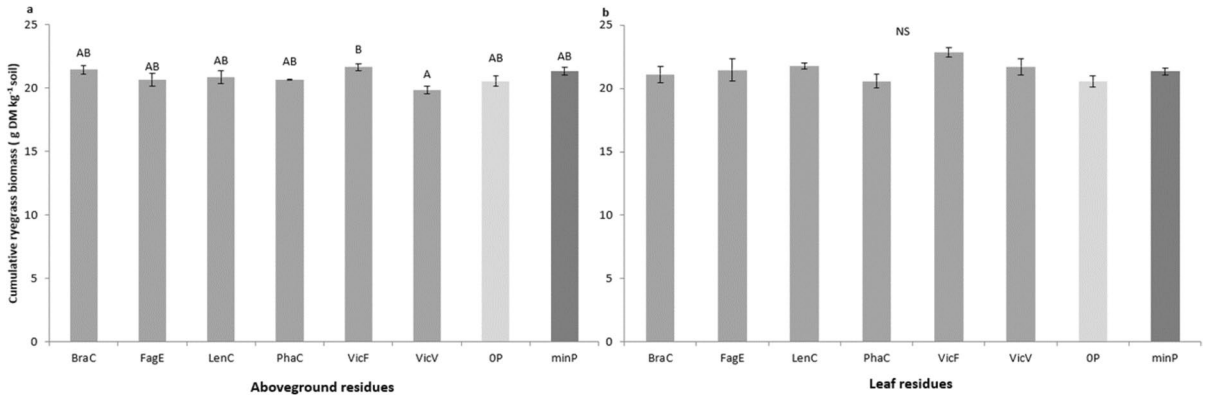

Fig. 1 Ryegrass biomass after amendment with cover crop residues. **a** Cumulative biomass after 110 days after amendment with aboveground residues; **b** Cumulative biomass after 110 days after amendment with leaf residues. ± standard error. Sum of 5 harvests. Letters above the bars represent statistically significant differences among treatments within the same residue type. *NS*—Not significant. BraC—*Brassica carinata*; FagE—*Fagopyrum esculentum*; LenC—*Lens culinaris*; PhaC—*Phacelia tanacetifolia*; VicF—*Vicia faba*; VicV—*Vicia villosa*; 0P—Control without P addition; minP——control with 15 mg P kg^{-1} mineral P

Significant differences in ryegrass biomass were observed for the first harvest, after which little to no differences were observed (Supplementary table S1).

The P nutrition index (indicative of a plant P limitation), averaged 50% across all harvests, decreasing over time from 53 to 43% on average. Average ryegrass P concentrations ranged between 2.9 and 0.6 g P kg^{-1}. The PNI differed significantly between treatments only until 70 days after amendment (Supplementary table S2). The NNI averaged at 91% across all harvests, first decreasing with time until 70 days after amendment (112% on average after 35 days, 77% on average after 70 days). The NNI increased thereafter (94% on average after 110 days) (Supplementary table S3). The PNI was not significantly correlated to ryegrass biomass for most harvests. The NNI was not correlated to ryegrass biomass until 70 days after amendment.

Effect of cover crop residues on ryegrass P uptake

Few significant differences between treatments were observed in total cumulative P uptake by ryegrass over the course of the experiment (Fig. 2). However, cover crop residues significantly affected ryegrass P uptake per cut (Supplementary table S4). Average ryegrass P uptake per cut decreased with time, from 7.7 mg P after 35 days to 5.0 mg P after 110 days. Cumulative P uptakes for residue amended treatments did not significantly differ from the unfertilized control, except for VicV aboveground residues. Amendments with aboveground residues of VicV resulted in a significantly lower cumulative ryegrass P uptake than the unfertilized control by 7.8 mg P (Fig. 2a). Ryegrass cumulative P uptakes were lower when amended with aboveground or leaf residues than equivalent mineral fertilization, except for FagE and VicF leaf residues (Fig. 2 a,b). Mineral fertilization increased ryegrass cumulative P uptake by 5.7 mg P, about a third of the 15 mg P applied.

Differences in P uptake mostly occurred in early harvests up to 70 days after amendment after which no significant differences were observed (Fig. 3). Thirty-five days after amendment most aboveground residues resulted in P uptakes that did not significantly differ from the unfertilized control (Fig. 3a). Moreover, most aboveground residues resulted in P uptakes significantly lower than equivalent mineral fertilization (Fig. 3a). Aboveground residues of VicV resulted in the lowest P uptake of all aboveground residues, significantly lower than unfertilized control after 35 days (Fig. 3a). Ryegrass P uptake after VicV aboveground residues amendment was 4.2 mg P lower than for the unfertilized control. After 70 days, ryegrass P uptake was significantly lower than for equivalent mineral fertilization for all aboveground residues except for FagE and PhaC residues (Fig. 3e). Significant differences in ryegrass P uptake were observed between residues 35 and 70 days after addition (Fig. 3a, e). After addition of leaf residues,

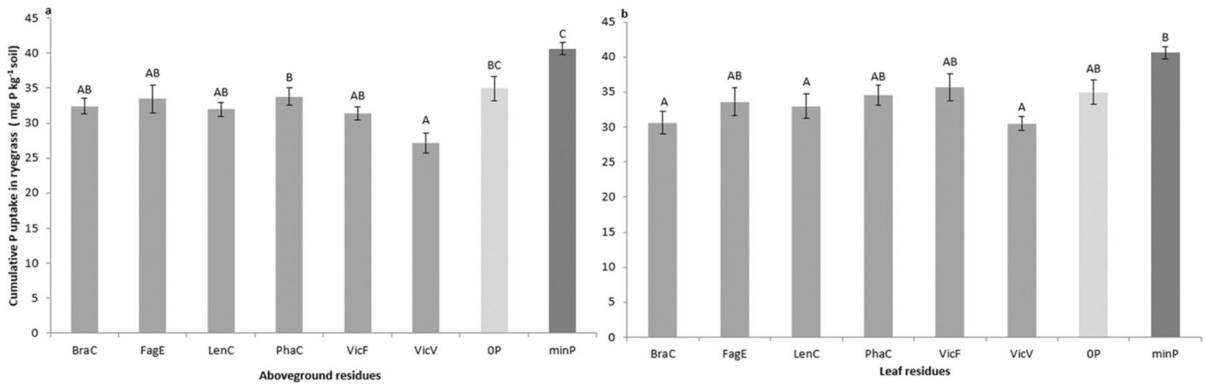

Fig. 2 Ryegrass cumulative P uptake after amendment with cover crop residues. **a** Cumulative P uptake over 110 days for aboveground residues; **b** Cumulative P uptake over 110 days for leaf residues. ± standard error. Sum of 5 harvests. Letters above the bars represent statistically significant differences among treatments within the same residue type. BraC—*Brassica carinata*; FagE—*Fagopyrum esculentum*; LenC—*Lens culinaris*; PhaC—*Phacelia tanacetifolia*; VicF—*Vicia faba*; VicV—*Vicia villosa*; 0P—Control without P addition; minP—control with 15 mg P kg^{-1} mineral P

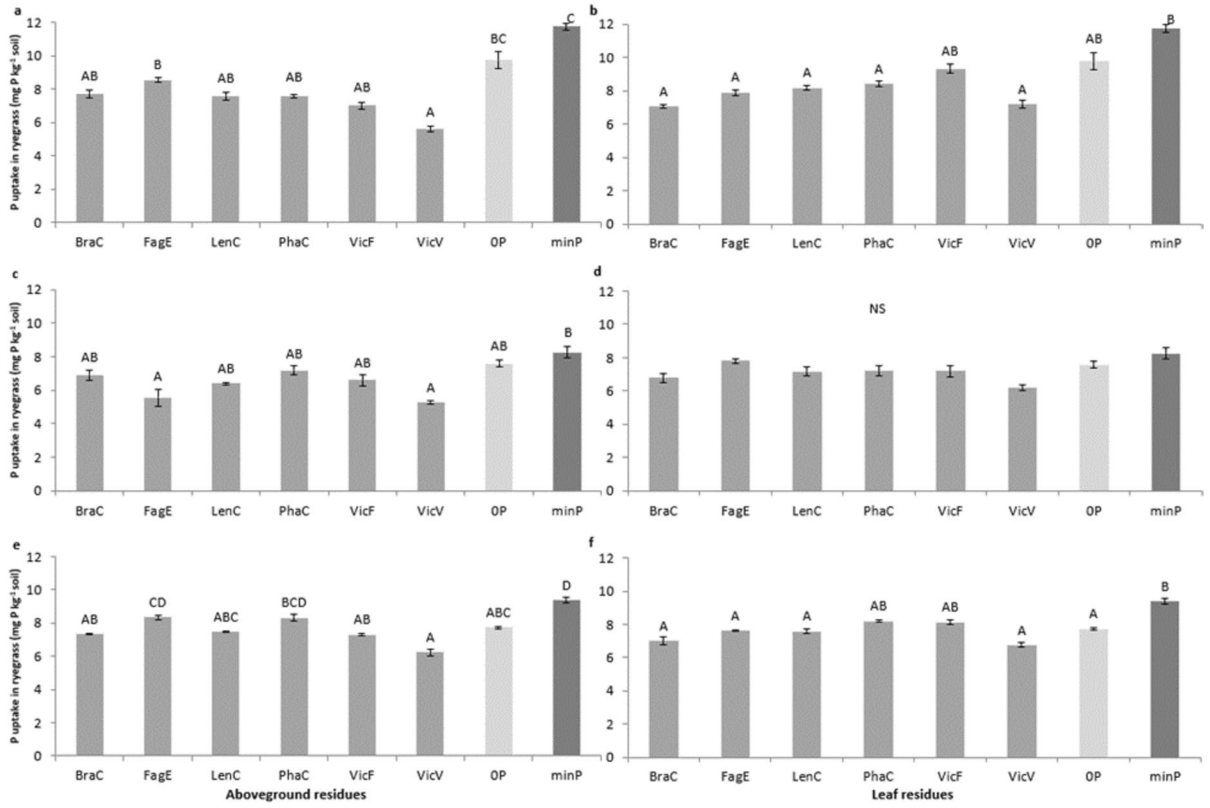

Fig. 3 Phosphorus acquired by ryegrass per harvest after amendment with cover crop residues. Ryegrass P uptake, mg P kg^{-1} soil. **a**, **b** P uptake 35 days after amendment of aboveground residues (left) or leaf residues (right); **c**, **d** P uptake 47 days after amendment of aboveground residues (left) or leaf residues (right); **e**, **f** P uptake 70 days after amendment of aboveground residues (left) or leaf residues (right). ± standard error. Letters above the bars represent statistically significant differences among treatments within the same residue type. BraC—*Brassica carinata*; FagE—*Fagopyrum esculentum*; LenC—*Lens culinaris*; PhaC—*Phacelia tanacetifolia*; VicF—*Vicia faba*; VicV—*Vicia villosa*; 0P—Control without P addition; minP—control with 15 mg P kg^{-1} mineral P

ryegrass P uptake did not significantly differ from the unfertilized control (Fig. 3b, d, f). Ryegrass P uptake after addition of leaf residues was significantly lower than the control with equivalent mineral fertilization, except for VicF and PhaC (Fig. 3a, c).

Effect of cover crop residues on soil P pools

Pools of soil P differed in their response to the addition of cover crop residues (Table 3). After 10 days, BraC or FagE aboveground residues addition increased P_{res} by 2.7 mg P kg^{-1} (BraC) and 3.2 mg P kg^{-1} (FagE) compared to the unfertilized control. LenC, PhaC and VicF aboveground residues addition did not significantly change P_{res} compared to the unfertilized control. Finally, VicV aboveground residues addition decreased P_{res} by 6.6 mg P kg^{-1} compared to the unfertilized control. Equivalent mineral fertilization increased P_{res} by 3.7 mg P kg^{-1} compared to the unfertilized control, about 25% of the P added.

Ten days after amendment most leaf residues resulted in similar P_{res} to the unfertilized control, although significant differences were observed between residues. FagE and VicF leaf residues resulted in significantly higher soil P_{res} than BraC and VicV residues. The effect of most leaf residues on P_{res} did not significantly differ from equivalent mineral fertilization.

Fifty-nine days after amendment aboveground residues did not significantly change P_{res} compared to the unfertilized control (Table 3). Mineral fertilization

Table 3 Soil resin extractable P content after amendment with cover crop residues

Residues	Time	BraC	FagE	LenC	PhaC	VicF	VicV	0P	minP
Aboveground	10	16.1 ± 0.4	16.6 ± 0.7	14.2 ± 1.1	15.2 ± 0.6	16 ± 1.1	6.8 ± 0.8	13.4 ± 0.6	17.1 ± 1.1
		C	C	BC	BC	ABC	A	B	C
	59	11.6 ± 1.1	14.1 ± 1.9	11.2 ± 0.7	14.4 ± 0.4	10.4 ± 1.5	9.4 ± 0.4	11.9 ± 0.6	14.7 ± 0.8
		AB	AB	AB	B	AB	A	AB	B
Leaf	10	11.1 ± 1.6	15.7 ± 0.3	14.3 ± 0.8	14.6 ± 1.2	16.5 ± 0.5	10.2 ± 0.9	13.4 ± 0.6	17.1 ± 1.1
		AB	C	AC	BC	C	A	ABC	C
	59	12 ± 0.7	15.3 ± 0.5	14.8 ± 1.1	14.7 ± 0.9	15.2 ± 0.5	13.8 ± 0.3	11.9 ± 0.6	14.7 ± 0.8
		A	B	AB	AB	AB	AB	A	AB

BraC—*Brassica carinata*; FagE—*Fagopyrum esculentum*; LenC—*Lens culinaris*; PhaC—*Phacelia tanacetifolia*; VicF—*Vicia faba*; VicV—*Vicia villosa*; 0P—Control without P addition; minP—control with 15 mg P kg^{-1} mineral P

Resin P in mg P kg^{-1} soil (± standard error). Time: Time point of extraction in relation to amendment. Letters represent statistically significant differences among treatments within the same residue type and time since amendment

similarly no longer significantly increased P_{res} compared to the unfertilized control. Significant differences were nonetheless observed between VicV and PhaC aboveground residues (P_{res} = 9.4 mg P kg^{-1} and 14.4 mg P kg^{-1} respectively). After 59 days, leaf residues similarly did not significantly change P_{res} compared to the unfertilized control, except for FagE leaf residues increasing P_{res} by 3.3 mg P kg^{-1}, 22% of the 15 mg P added. Significant differences between residues were also observed, with a lower P_{res} after BraC leaf residues (12.0 mg P kg^{-1}) than after FagE leaf residues (15.3 mg kg^{-1}).

Amendment with aboveground residues of VicV resulted in the highest P_{mic} after 10 days, significantly higher than the unfertilized control and BraC residues and PhaC residues (Table 4). The increase of P_{mic} after the addition of aboveground residues of VicV was of 9.2 mg P kg^{-1} compared to the unfertilized control, about two third of the P added in residues. Microbial P significantly decreased overtime on average for all residue treatments, from 16.2 mg P kg^{-1} after 10 days to 8.6 mg kg^{-1} after 59 days.

Isotopic ^{33}P labelling

No significant SA dilution was observed in ryegrass between the unfertilized control and soil amended with 15 mg P kg^{-1} mineral fertilizer at any time point, not allowing us to calculate P derived from fertilizer via indirect labelling (Supplementary figure S5). Likewise, SA in ryegrass for the unfertilized control did not significantly differ from any residue treatment at any time point (Supplementary figure S6). Similarly, SA in resin extracts and microbial extracts did

Table 4 Soil microbial P content after amendment with cover crop residues

Residues	Time	BraC	FagE	LenC	PhaC	VicF	VicV	0P	minP
Aboveground	10	12.6 ± 2.1	16.2 ± 0.9	14.6 ± 1	13 ± 1.6	14 ± 2,9	21.1 ± 0.3	11.8 ± 1.8	14.2 ± 2.1
		A	AB	AB	A	AB	B	A	AB
	59	10.7 ± 4.5	9.8 ± 1.3	8.3 ± 1	5.5 ± 1.6	7.8 ± 1.2	6.2 ± 1.7	9.3 ± 1.7	10.7 ± 2.1
		NS							
Leaf	10	19.9 ± 3.5	15 ± 2.2	16.8 ± 3	14.3 ± 2.8	16 ± 1.3	20.8 ± 1	11.8 ± 1.8	14.2 ± 2.1
		NS							
	59	7.6 ± 4	8.8 ± 1.2	7.1 ± 1.3	8.1 ± 2	11.7 ± 2	8.7 ± 2	9.3 ± 1.7	10.7 ± 2.1
		NS							

BraC—*Brassica carinata*; FagE—*Fagopyrum esculentum*; LenC—*Lens culinaris*; PhaC—*Phacelia tanacetifolia*; VicF—*Vicia faba*; VicV—*Vicia villosa*; 0P—Control without P addition; minP—Control with 15 mg P kg^{-1} mineral P

Mic P in mg P kg^{-1} soil (± standard error). Time: Time point of extraction in relation to amendment. Letters represent statistically significant differences among treatments within the same residue type and time since amendment

not significantly differ between the unfertilized control and the control amended with 15 mg P kg^{-1} mineral fertilizer at any time point.

Direct labelling control indicated Pdff ranging from 1.1 mg P in harvest 1 after 35 days to 0.3 mg P in the fifths harvest after 110 days. Cumulative Pdff in ryegrass shoots was 3.0 mg P, 20% of the 15 mg P applied (Supplementary table S7).

Relation between residue traits and P uptake

Cumulative P uptake in ryegrass over the course of the experiment was correlated positively with residue P concentration (Pearson correlation coefficient r = 0.96, $p < 0.001$) and negatively with residue C:P (Pearson correlation coefficient r = − 0.97, $p < 0.001$) (Supplementary figure S8). Residues' C:N, N:P and N and C concentrations as well as SLA were not correlated with cumulative P uptake by ryegrass (Supplementary figure S8). Similar positive correlations with residue P concentration and negative correlations with residue C:P were observed for soil P pools (Supplementary figure S9). Residues' C:P was the single best predictor for both cumulative P uptake and per harvest P uptake in ryegrass (Supplementary table S10). Negative relationships between residue C:P and P uptake by ryegrass were observed until 70 days after addition (Fig. 4). VicV aboveground residues with a C:P ratio above 300 contributed to overall model fit, while excluding this residue still resulted in significant negative relationships between C:P and P uptake by ryegrass. The slope coefficient of the linear models decreased in absolute value from − 0.016 35 to − 0.011 on day 70. Incorporating incubation results in the model improved fit. Soil P_{res} was positively correlated with cumulative P uptake by ryegrass (Spearman correlation coefficient rs = 0.66, $p < 0.024$ after 10 days, Pearson correlation coefficient r = 0.81, $p = 0.001$ after 59 days, supplementary figure S11). Soil P_{mic} 10 days after amendment was negatively correlated with cumulative P uptake by ryegrass (Pearson correlation coefficient r = − 0.64, $p = 0.019$). Best model fit was achieved with residue C:P and P_{res} as predictors (Supplementary table S10).

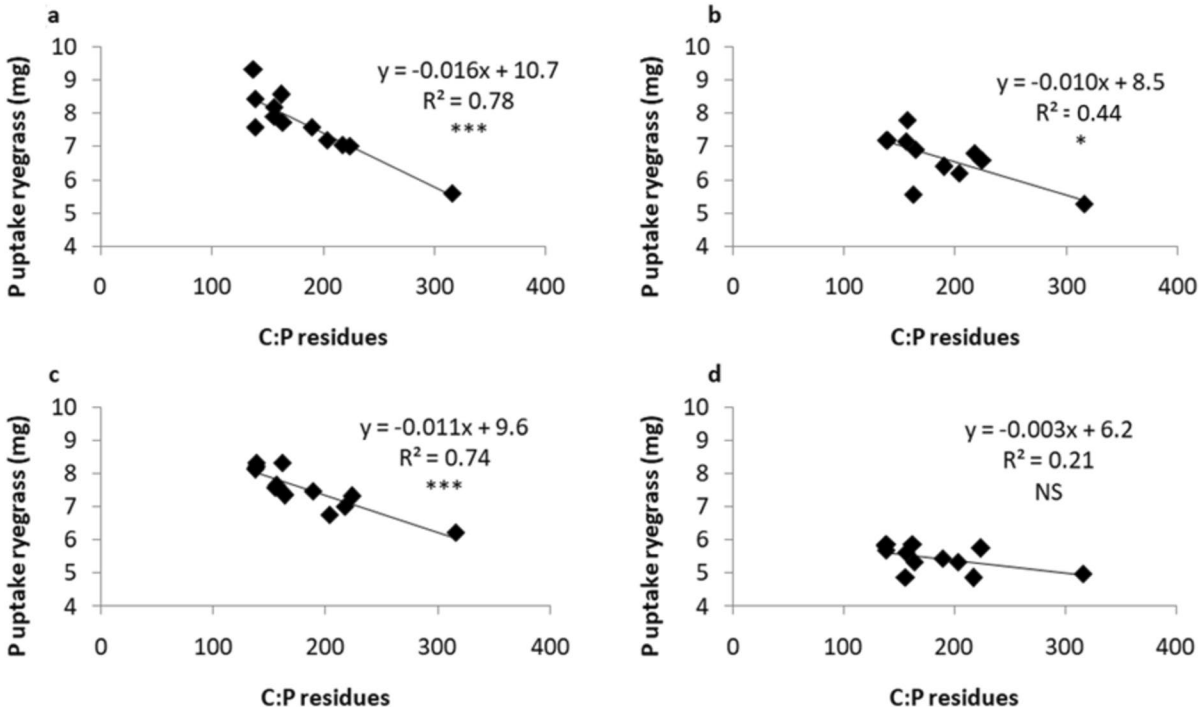

Fig. 4 Relationship between P uptake in ryegrass and cover crop residues C:P ratio. **a** 35 days after amendment; **b** 47 days after amendment; **c** 70 days after amendment; **d** 91 days after amendment. No significant model after 91 days onward after amendment

Discussion

Soil P availability changed substantially shortly after residue incorporation

The role of cover crop residues on P availability and subsequent crop P uptake remains unclear (Nachimuthu et al. 2009; Noack et al. 2012; Thibaud et al. 1988). Over the course of four months, our experiments highlighted contrasted effects of cover crop residues with diverse traits, converging with time toward mostly neutral effects on subsequent crop P uptake. Shortly after cover crop incorporation, large changes in soil P availability were observed, ranging from + 24% to − 49% P_{res} compared to the unfertilized control. The incorporation of most crop residues at first resulted in lower ryegrass P uptakes than equivalent mineral fertilization. Decreased P uptakes were especially pronounced for *Vicia villosa* (VicV) residues, decreasing ryegrass P uptake by 43% compared to the unfertilized treatment. *Vicia villosa* residues were previously observed to contribute less to P uptake compared to other residues (Maltais-Landry and Frossard 2015). Alongside other residue properties, *Vicia villosa* phenols and secondary metabolites content have been proposed to possibly slow its decomposition and nutrient release (Gougoulias 2011; Maltais-Landry and Frossard 2015). While less pronounced with time, these early dynamics had lasting effects on cumulative P uptakes, likely due to the importance of P availability in early stages of growth (Grant et al. 2001).

Short term effects on soil P pools faded with time

Early decreases in P availability and ryegrass P uptake rapidly became less pronounced with time, alongside a gradual decrease in P_{mic} for all organic treatments. Coupled with an increase in P_{mic} of 79% for *Vicia villosa* residues compared to the unfertilized control, our results strongly suggest microbial P immobilization, which is consistent with other studies on crop residues (Alamgir et al. 2012; Noack et al. 2014; Traoré et al. 2020). Decreases in P availability induced by microbial P immobilization are expected to fade with time due to microbial turnover releasing immobilized P in plant available forms (Bünemann et al. 2004; Oehl et al. 2001). However, we did not observe increases in soil P availability (P_{res}) after 59 days despite decreases in microbial P over the same period. As suggested in Alamgir et al. (2012) early neutral effects could have resulted from a balance between residue P release and microbial P immobilization for residue barely supplying enough P to meet microbial requirement during residue degradation. Later release of microbial P in plant available forms could then have been prevented by quick transformation of microbe-derived P to more stable, less available P forms (Alamgir et al. 2012; Ha et al. 2007). Strong sorption of P derived from microbial turnover is unlikely in our soil with an average spike recovery of 58% after 16 h. However, recovery of mineral fertilizer was considerably less, 20% of applied P based on direct labelling control. The soil used in the experiment presented both a low Olsen P and HCl extracted P in comparison to a significantly higher total P stock, prompting further investigation of the diverse P pools present and their role in sorption dynamics. Further efforts would be needed to investigate the fate of turnover microbial P and its long-term contribution to P availability.

Residue P and C:P mediated changes in P availability

Diverse chemical and morphological residue traits can offer insight into cover crops uncertain effects on subsequent crop P uptake (Espinosa et al. 2017; Hallama et al. 2019; Maltais-Landry and Frossard 2015). However, inconsistent and potentially misleading effects are reported for some chemical traits such as residue P concentration or residue C:P ratio (Damon et al. 2014; Kwabiah et al. 2003; de Oliveira et al. 2017; Umrit and Friesen 1994). Out of the traits examined, we highlighted strong to moderate correlations between residue C:P ratio and ryegrass P uptake up to 70 days after amendment ($R^2 = 0.8$ after 35 days; $R^2 = 0.4$ after 47 days, $R^2 = 0.7$ after 70 days). A strong influence of residue C:P ratio was also observed on soil P availability. In agreement with reports of decreased P uptake by crops amended with residue with a C:P ratio > 300 (Tate 1985), we observed decreases of ryegrass P uptake of up to 43% compared to the unfertilized control for *Vicia villosa* residues with a C:P of 314. Microbial P immobilization was reported to occur after amendment with residue with C:P ratio > 200 in Alamgir et al., (2012), while Prescott (2005) reported a range of critical C:P ratios from 230 to 480. Linear models predicting P

uptake by ryegrass showed poor fits for C:P ratios lower than 190 in our experiment, consistent with a C:P threshold for P microbial immobilization of around 200. However, increases in P availability were observed after addition of BraC residues (C:P 164, 2.44 g P kg^{-1}) or FagE residues (C:P 162, 2.49 g P kg^{-1}), suggesting a higher critical C:P ratio for quick mineralization and release of residue P than the C:P ratio of 100 suggested in Alamgir et al., (2012) or Hansen et al. (2022).

Residues increasing soil P availability in the short term in our experiment nonetheless had a P concentration close to 2.5 g P kg^{-1}, concomitant with suggested thresholds of 2.5 to 3 g P kg^{-1} for fast P release from residues (Damon et al. 2014; Hallama et al. 2019; Maltais-Landry and Frossard 2015). Overall, despite the transitory nature of C:P ratio influence on P availability via microbial P immobilization, C:P ratio was strongly correlated with the cumulative P uptake in ryegrass over the four months of our experiment. Our results thus strengthen the proxy offered by cover crop residue C:P ratio in understanding subsequent plant P uptake in our moderately P limited soil.

Recent efforts similarly highlighted the role of cover crop residues C:P ratio for P uptake by subsequent crops. Although they did not quantify it, previous authors suggested that residue C:P ratio drive P immobilization dynamics (Hansen et al. 2022). Our results reinforce this central role of residue C:P ratios and confirm important microbial P immobilization dynamics. However, different effects of residue C:P ratio were reported according to soil P status in Hansen et al. (2022). This prompts further efforts to investigate the complex impacts of soil P status on the influence of residue C:P on P release from residue via microbial dynamics.

Neutral effects of residue specific leaf area

Morphological traits and architecture of plant tissues, such as SLA, have also been observed to play an important role in residue decomposition and nutrient dynamics across natural ecosystems (Garnier et al. 2004; Liu et al. 2018; Perez-Harguindeguy et al. 2013; Santiago 2007; Zukswert and Prescott 2017). Litter physical traits, such as SLA, have been proposed to modulate nutrient release and residue decomposition rate (Zukswert and Prescott 2017). Reduced surface area per mass, associated with lower SLA, was for instance proposed to explain slower residue decomposition and P release in larger residue pieces (Noack et al. 2014). Recent efforts moreover suggested that different tissue architectures may explain moderate rather than high correlations between C:P and P uptakes in a decomposition experiment (Hansen et al. 2022). Contrary to our hypothesis, no correlation was observed between residues SLA and P uptake by ryegrass or soil P availability. These results contrast with findings from natural ecosystems (Liu et al. 2018; Santiago 2007). The lack of correlation between SLA and leaf P concentration or other residue traits in our experiment may explain these results. As cover crop species are primarily selected for their fast growing characteristics and easy termination (Hallama et al. 2019), the common cover crop species used might have restricted the range of SLA values compared to more wide variation in natural ecosystems, resulting in no visible effect. Reduced decomposer abundance, due to soil sieving and long soils storage prior to the experiment, likely also reduced potential effects of SLA via modulating decomposer access to residues. Overall, here, residue SLA did not offer a proxy to understand and possibly model the contribution of cover crop residues to P availability.

Implications for cover crop contribution to subsequent crop P uptake

Potential benefits of cover cropping on P availability have been reviewed across a wide range of contexts with reported increases of up to 50% in P uptake in subsequent crops (Hallama et al. 2019). However, cover cropping benefits remain very inconsistent with reports of positive but also negative or neutral impacts on subsequent crop P uptake (Hallama et al. 2019). Our results showed similar cumulative P uptake after crop residue amendment as compared to the unfertilized control, with little differences between species except for *Vicia villosa*. Comparable observation of little differences between vegetative stage residues were previously reported by Maltais-Landry and Frossard (2015), while other studies highlighted more pronounced differences (Eichler-Löbermann et al. 2008; El Dessougi et al. 2003). An early decrease or at best maintained P availability in amended soils in our experiment contrasts with reports of increased P

availability and uptake after cover crop residues amendment in Maltais-Landry and Frossard (2015), and recent reports of low but positive effects on subsequent P uptake (Hansen et al. 2022). Based on cumulative uptakes over four months, the change in P uptake in ryegrass in our experiment ranged from an increase of + 2% for VicF leaf residues to a decrease of 22% for VicV aboveground residues compared to the unfertilized control. However, no significant isotopic dilution was observed between our minP and unfertilized control in ryegrass biomass, as well as in resin extracts. We could thus not calculate P derived from residues based on indirect isotopic labelling but only based on difference in P content in the different components of our system (ryegrass, soil resin extractable P, and microbial P).

Our cover crop residues had lower P concentrations (between 1.3 and 3 mg P kg^{-1}) and higher C:P ratio (between 137 and 316) compared to other studies (Hansen et al. 2022; Maltais-Landry and Frossard 2015), likely because of moderate P limitation during growth. Lower P concentrations and higher C:P ratio for our residues could have resulted in microbial P immobilization and simultaneous P mineralization and release balancing out as proposed in Alamgir et al. (2012). Balanced microbial P immobilization and simultaneous P release would then explain mostly neutral effects of cover crop residues on ryegrass P uptake. Lower contribution of residue P to P uptake by ryegrass in our experiment likely also resulted from our lower application rate of 15 mg P kg^{-1} soil compared to 50 mg P kg^{-1} soil in Hansen et al. (2022). Our application rate corresponded to a field productivity of 6.5 t DM ha^{-1} and a residue P concentration of 3 mg P kg^{-1} which are representative of what would be expected in the fields sampled. However, cover crop biomass production and P concentrations may vary greatly according to species, growth period, climatic conditions and nutrient status of the soil. Our low but relevant P dose added could have contributed to the lack of significant isotopic dilution. Indeed, other studies using the same indirect isotope method for determining the P uptake by plants applied higher P rates of e.g., recycling fertilizers (Brod et al. 2016, 30 mg P kg^{-1} soil; Nanzer et al. 2014 50 mg kg^{-1} soil), or animal manure (Oberson et al. 2010 30 mg P kg^{-1} soil), or plant residues (Hansen et al. 2022, 50 mg kg^{-1} soil). The combination of microbial immobilization and slow release of small amounts of remineralized microbial P may have been too low for detection.

Co-limitation with N (average PNI = 50%, average NNI = 91%) might have also amplified the reduced P uptakes as treatments with higher NNI tended to have lower PNI in early harvests, despite important N inputs of 120 mg kg^{-1} soil supplied after each harvest (600 mg kg^{-1} soil over the course of the experiment). Moderate P limitation may also have influenced the potential contribution of residue P to P plant uptake. Higher contribution of residue P to P availability is indeed most likely to occur in contexts with high P availability where cover crop accumulate large amount of P (Damon et al. 2014; Thibaud et al. 1988). The contribution of cover crops to P availability was even proposed to be agronomically significant only in contexts where cover crop accumulate large amount of P (Damon et al. 2014; Thibaud et al. 1988). Maintained or decreased subsequent crop P uptake in our soil with moderate P limitation may suggest that benefits observed in similar contexts (Hallama et al. 2019) may be related to cover crops capacity to forage for P unavailable to the main crops, and to increase P availability via mobilization during growth and reduce loss (Hallama et al. 2019). Optimizing cover cropping benefits for P availability in moderately P limited soil may also be ensured via agricultural practices either avoiding the temporary decrease in P availability via carefully managing cover crop C:P ratios via early termination or adapted composition or enhancing P availability during decomposition via mineral fertilization (Baggie et al. 2005).

Conclusions

Understanding the factors involved in the fate of residue P in the plant-soil-microbe system is central to manage and improve soil P availability in cropping systems. Our study reinforced the role of residue C:P ratio, strongly underlining the importance of microbial P immobilization dynamics when adding C as part of residues. Residues with high C:P ratios (i.e., > 300) resulted in decreased P availability, by up to 49% for resin P extracts, and decreased cumulative P uptake by up to 22% compared to the unfertilized control. Contrary to our hypothesis, residue morphology via the proxy of SLA did not appear to affect P release. Our results highlighted mostly neutral effects

of cover crop residues grown in a moderately P limited soil on P availability and P uptake by a subsequent crop, which contrasts with previous studies often reporting positive results. Neutral effects of P release from cover crop residues on soil P availability suggested that in context with moderate P availability cover cropping benefits for P availability may be achieved via other pathways such as P mobilization by cover crops during growth, shifts in microbial communities under cover cropping or reduced losses. Exploring the relative contribution of these pathways relative to P release from residue in contexts with varied soil P availability could provide important insights into optimal cover crop composition for enhanced P availability in cropping systems.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose. The authors have no conflict of interests to declare that are relevant to the content of this article.

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