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REGULAR ARTICLE

The role of microbes in the increase of organic phosphorus availability in the rhizosheath of cover crops

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Abstract

Background and aims The characterisation of plant-available phosphorus (P) pools and the assessment of the microbial community in the rhizosheath of cover crops can improve our understanding of plant–microbe interactions and P availability.

Methods Mustard (*Sinapis alba*), phacelia (*Phacelia tanacetifolia*) and buckwheat (*Fagopyrum esculentum*) were grown as cover crops before soybean (*Glycine max*) in an on-farm experiment on a soil low in available P in southwest Germany. The cycling of P through the cover crop biomass and the enzyme-availability of organic P (P_{org}) pools in the cover crop rhizosheath were characterised. The soil microbial community (PLFA), activity (acid and alkaline phosphomonoesterase, as well as phosphodiesterase), and microbial P were assessed. The abundance of 16S-rRNA and *phoD*, coding for

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alkaline phosphomonoesterase in bacteria, were quantified using real-time qPCR.
Results Mustard contained the greatest amount of P in its large biomass. In the rhizosheath of all cover crops, the concentration of enzyme-labile P_{org} was higher than that in the control bulk soil, along with substantial increases of microbial abundance and activity. There were little differences among cover crop species, few changes in the bulk soil and only a limited carryover effect to soybean, except for fungi.
Conclusions Turnover of microbial biomass, especially saprotrophic fungi, increased by rhizodeposition of cover crop roots; this was likely responsible for the observed increases in enzyme-available P_{org}. Microbial function was correlated linearly with microbial biomass, and the data of enzyme activity and *phoD* did not suggest a difference of their specific activity between bulk and rhizosheath soil.

Keywords Plant–microbe interactions · Rhizosphere · P mobilisation · Enzyme Addition Assay · PLFA · Nutrient management

Introduction

Conventional agricultural management requires a rethinking to cope with externalities including environmental pollution, soil degradation, biodiversity loss and the exhaustion of mineable reserves of fertilisers (IAASTD 2009). Of great concern is the transgression of the planetary boundaries of nutrient cycles, with phosphorus (P) being one of the most prominent issues (Campbell et al. 2017). Agricultural production is the main driver of the global P cycle, and overapplication of P fertilisers led in rich countries to the accumulation of legacy P in many agricultural soils (Nesme and Withers 2016).

Soil P is present in different inorganic (P_i) and organic (P_{org}) P pools of varying degrees of availability; therefore, the needed solutions are complex and require fundamental changes of the agricultural system. The adoption of agroecological farming techniques such as cover cropping provides an opportunity of a step in the right direction (Altieri 2018). Cover crops have potential for P management, reducing environmental hazards in systems with high P loads, and improving crop P nutrition in soils with low P availability (Oberson et al. 2006; Simpson et al. 2011).

The use of cover crops can potentially alter soil P dynamics and the main crop may benefit by different mechanisms. These include uptake, storage and subsequent mineralisation of P from cover crop litter (*plant biomass pathway*), mobilisation of otherwise unavailable soil P pools via biochemical modification of the rhizosphere (*biochemical pathway*), and an increased capacity of the soil microbial community to cycle P (*microbial pathway*) (Hallama et al. 2019; Soltangheisi et al. 2020; Boselli et al. 2020). Especially the soil–plant-microbe feedback is complex and heavily influenced by several site-specific factors including soil type and climate as well as agricultural management, for example, cropping sequence and fertilization regimes. In addition, in the case of cover crops, the plant species used, their root architecture and biomass (Kim et al. 2020). Root exudates and –deposits are quickly used by microbes as C-source, increasing microbial abundance, and they shape the composition of the microbial community in the rhizosphere (*rhizobiome*). The enhanced microbial activity, together with accumulation of P in living microbial biomass or dead cells (*necromass*) (Hinsinger et al. 2011) increases microbial nutrient cycling and can therefore be considered as a major trigger for soil – plant – microbe feedbacks (Jacoby et al. 2017).

Cover crop species differ in their P uptake and effect on the soil (micro-)biology and chemistry, and therefore in their potential to contribute to a P benefit to the main crop. Some plants, for example, buckwheat (*Fagopyrum esculentum*), mobilise poorly-mobile P_i pools (Schelfhout et al. 2018). Mustard (*Sinapis alba*), a member of the Brassicaceae family, produces a large biomass with a high P concentration; a high rhizosheath phosphatase activity is thought to be part of its P-acquisition strategy (Hunter et al. 2014). Other species such as phacelia (*Phacelia tanacetifolia*), form mycorrhizas and their very fine root system is expected to interact strongly with the soil microbial community (Eichler-Löbermann et al. 2009).

Since plant P nutrition depends not only on their own P-acquisiton strategies, but also on the potential of microorganisms to moblise P from different inorganic and organic sources, there is a need to study microbial-driven processes leading to mineralisation of P_{org} pools (George et al. 2018). The

outlined three pathways of cover crop-derived P benefits always occur simultaneously. However, the relative importance of each pathway depends on multiple factors such as cover crop species and growth, as well as the soil microbial community and P pools. In the past, soil chemistry dominated agricultural sciences and plant nutrition studies, with much less attention for the role of microorganisms in cycling of P_{org} (Johnston and Bruulsema 2014). Information about the potential activity of phosphomonoesterases and phosphodiesterase as well as the quantification of P_{org} is available (Nuruzzaman et al. 2006; Maltais-Landry et al. 2014), while the inclusion of molecular tools to assess the genomic background of the microbiota that drive P_{org} transformation is still rare (Ragot et al. 2017; Fraser et al. 2017).

The rhizosheath is the agglutination of soil particles around the roots, and is biologically the most active fraction of the rhizosphere (Ndour et al. 2020). Therefore, in addition to standard soil analyses, assessment of the rhizosheath under field conditions may allow us to improve our understanding of how cover crop-microbial interactions affect the ecophysiology of P dynamics, also regarding the persistence of these changes over time for the subsequent main crop.

The present study aimed to address the question of whether the availability of P_{org} pools in the soil can be increased by cover crops, in particular regarding the relevance of the enhancement of the abundance and activity of microbes in the rhizosheath. These insights could improve our understanding of underlying mechanisms regarding the potential of P mineralisation as one of the main processes for the supply of P to plants. An additional aim was to elucidate whether the observed changes of the microbial community persist in the soil and can still be detected in the rhizosheath of the following main soybean crop, a legume with a moderate capacity for P-acquisition (Belinque et al. 2015; Lyu et al. 2016). These questions were resolved by characterising the lability of soil P_{org} pools for phosphatases (Jarosch et al. 2019) in the cover crop rhizosheath. Further, we assessed the role of the different microbial groups as important sources for the activity of P-cycling enzymes, and quantified *phoD*, a gene coding for alkaline phosphomonoesterase in bacteria.

The present study aimed to test the following hypotheses:

The selected cover crops increase labile P_{org} derived from microbial necromass or rhizodeposition in their rhizosheath;
Cover crop species differ in their plant–microbe interactions, leading to a distinct microbial community and activity in their rhizosheath;
The cover crops shape their rhizobiome towards an increase in beneficial functions, e.g., by enhancing the specific enzymatic activity per unit of microbial abundance;
Soybean as a subsequent main crop benefits from the increase in labile P_{org} and microbial activity by the cover crops.

Materials and Methods

Site description

An on-farm field experiment was conducted in 2016–2017 near Wendelsheim in southwest Germany (48.5111°N, 8.9197°E). The soil is a Regosol in an region of loess-derived soils (IUSS Working Group WRB 2015; Regierungspräsidium Freiburg, Landesamt für Geologie, Rohstoffe und Bergbau 2020) and has a clayey loam texture with a $pH_{(CaCl2)}$ of 7.4 and a soil organic carbon concentration of 18 g kg^{-1} in 0–20 cm. The climate is temperate with a mean annual temperature of 8.8ºC and 839 mm precipitation (monitoring station Wetterstation Unterjesingen, 5.6 km from the site). The field has been managed by a farmer under no-till management for around 12 years and without applying any P-containing fertilizers for the last 20 years. As a consequence of stratification, soil organic matter (including P_{org}) accumulated in the topsoil, while available P_i was probably depleted over the years. Soil P availability was low for the region, with an average content of resin-extractable P of 16 µg g^{-1} at the beginning of the experiment. Contrary, total P concentration was with 1012 mg kg^{-1} rather high.

Experimental design

The crop rotation for the experiment was spring barley (*Hordeum vulgare* var. *Avalon*)– cover

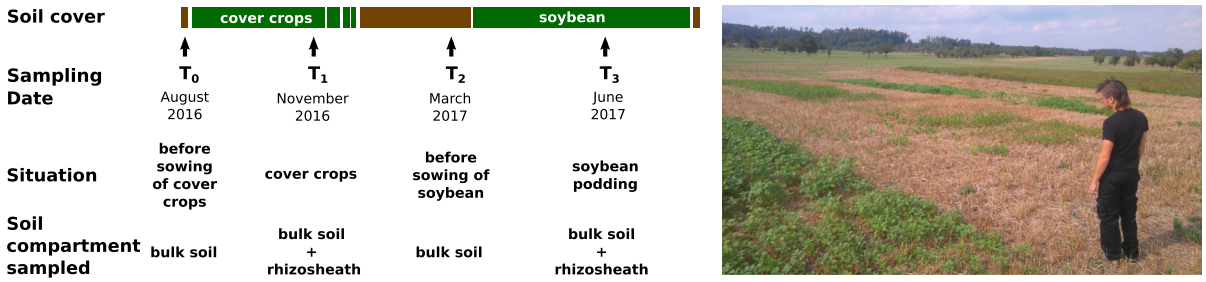


Fig. 1 Soil cover, sampling scheme and a view of the field experiment

crops– soybean (*Glycine max* var. *Tourmaline*). Fertilisers were not applied during the course of the experiment. An overview of soil cover and sampling dates is presented in Fig. 1.

The cover crops were grown in plots of 8 m by 50 m in four randomised complete blocks (in total 16 plots). Four cover crop treatments were established: *Fagopyrum esculentum* (buckwheat), *Sinapis alba* (mustard) and *Phacelia tanacetifolia* (phacelia) were direct seeded with a row distance of 16 cm in August 2016 after harvesting the wheat, while the fallow treatment was left bare to serve as control. Representing a common practice among farmers in the region, the selected cover crops died at the start of the winter frosts in November/December. Consequently, the cover cropped plots were also fallow until soybean was sown in March 2017, albeit covered by the plant litter.

Soil samples were taken at the following times: August 2016, before seeding the cover crops, November 2016, at the end of the growing period of the cover crops, March 2017, before seeding the soybean crop, and June 2017, in the full soybean stand. In November 2016 and in June 2017, in addition to the bulk soil samples, the cover crop and soybean rhizosheaths were sampled. As, by definition, there were no plants in the bare fallow plots in November, there was no rhizosheath sampling in the control treatment at this time. Later, in June, when soybean was growing on all plots, its rhizosheath was sampled in all treatments.

Bulk soil samples were taken at 0–10 cm depth with an auger from around six locations inside each plot. For the rhizosheath sampling, 5–10 vigorous plants, depending on the size of the rooting system, were selected from each plot and dug out in a 25 × 25 × 10 cm block together with their intact roots and transported to the laboratory. The same day, the roots were gently separated and the attached soil (0–10 mm distance to the root) was removed with a toothbrush, resulting in rhizosheath samples. All soil samples were sieved at 5 mm and stored at -20ºC until analysis.

Plant biomass and P content

The roots and shoots of the cover crop plants sampled for their rhizosheath were separated and dried (60ºC for 72 h). The biomass of both compartments was determined and subsamples were ball-milled for the analysis of C, P and N. Soybean grains were collected after harvest and also analysed for C, P, N by dry combustion coupled with an Elemental Analyser or ICP-OES, respectively (VDLUFA 1995a, b). Due to a communication problem, the soybean yield could not be quantified.

Enzymatic availability of organic P pools

To characterise different P_{org} forms according to their lability for enzymatic degradation, an enzyme-addition assay was used (Bünemann 2008; Jarosch et al. 2015). The assay consists of adding substrate-specific enzymes to soil NaOH/EDTA-extracts to hydrolyse specific P_{org} compounds. The increase in P compared with a control sample without added enzymes corresponds to the enzyme-labile P_{org} pool in the extract.

Organic P was defined as the difference between total P (P_t) after wet digestion with persulfate (Bowman 1989), and molybdate-reactive P (Ohno and Zibilske 1991) in the NaOH/EDTA extract. Although the residual, molybdate-unreactive P may also include other (inorganic) P compounds (Gerke 2010), for the sake of simplicity, we considered it P_{org}.

For the measurement of the enzymatic availability of P_{org}, the oven-dried (60ºC for 72 h) and milled soil samples were extracted following a ^{31}P-NMR extraction protocol (Bowman and Moir 1993), shaking for 16 h with 0.25 M NaOH and 0.05 M EDTA using a soil:extractant ratio of 1:10 (w/v). The suspensions were centrifuged for 10 min (2000 g) and filtrated (Whatman grade 40, ash-free paper). The extracts were transferred to transparent 96 well microplates, adding substrate-specific phosphatases and MES buffer adjusted to pH 5.2 in a final volume of 300 µl per well. Four distinct enzymes were used: 20 µl acid phosphatase (Sigma P1146, Sigma-Aldrich, St. Louis, USA: 50 units diluted in 15 ml H_2O) alone or together with 20 µl nuclease (Sigma N8630, Sigma-Aldrich, St. Louis, USA; 0.167 mg diluted in 1 ml H2O), or 40 µl of a fungal phytase (*Peniophora lycii*, Ronozyme NP, Novozyme, Bagsværd, Denmark). The enzymes were added to wells containing 40 µl NaOH-EDTA extract and MES buffer adjusted to pH 5.2 with a concentration of 0.2 M in the final volume of 300 µl per well. All reagents were prepared with autoclaved H_2O. The plates were incubated for 24 h at 37ºC, while being horizontally shaken at 40 rpm. For the detection of released P, aliquots of 25 µl were transferred to another plate with 175 µl of H_2O and 50 µl of malachite I in each well (Ohno and Zibilske 1991). After 10 min, 50 µl of malachite II was added and the absorbance was measured at 610 nm (HTX Synergy, BioTek Instruments, Winooski, USA). For each sample, three analytical replicates were analysed in separate runs.

The addition of acid phosphomonoesterase alone hydrolysed non-phytate-monoesters, for which the term "monoesterase-labile P_{org}" is used. The addition of phosphodiesterase/nuclease mineralised "diesterase-labile P_{org}". Since phosphodiesterase hydrolyses only the first of the two ester bonds in diesters, the combination with phosphomonoesterase was required to produce detectable phosphate. As preliminary tests revealed that the fungal phytase acts also as unspecific phosphomonoesterase and mineralises non-phytate monoesters, the monoesterase-labile P_{org} pool had to be subtracted from the phosphate released by the phytase to obtain the "phytase-labile P_{org}" pool. As each of the four field replicates of each treatment was analysed in three separate runs, the individual analysis run was included as a random effect when averaging the analytical replicates.

Microbial biomass P

Phosporus in the microbial biomass (P_{mic}) was determined in fresh soil by hexanol fumigation and simultaneous extraction with anion exchange resin membranes (Kouno et al. 1995). For that, 2.5 g dry weight of frozen soil was extracted with 20 ml deionised H_2O and two resin strips that were charged with 0.5 M $NaHCO_3$. Subsamples received either no treatment (P_{resin}), 1 ml of 1-hexanol (P_{hex}) or 1 ml of a solution with a known P content (P_{spike}) equal to 25 mg P kg^{-1} soil. Samples were shaken horizontally for 16 h at 150 rpm. Thereafter, the resins were transferred to another vial, shaken for 1 h with 1 M HCl to desorb the P from the resins and the orthophosphate-P concentration was measured colorimetrically at 610 nm (Murphy and Riley 1962). Microbial biomass P was calculated by

$$P_{mic} = \frac{P_{hex} - P_{resin}}{P_{spike} recovery}$$

where P_{spike} recovery is the fraction of a recovered P spike compared with the untreated P_{resin} subsample. The P_{spike} recovery was calculated for each sampling date and soil compartment separately, ranging between 31 and 63%. A K_P-conversion factor to account for incomplete extraction of microbial P was not applied, since it was not determined for this specific soil (Brookes et al. 1982; McLaughlin et al. 1986).

Potential activity of extracellular enzymes

Potential activities of acid phosphomonoesterase (EC 3.1.3.2), alkaline phosphomonoesterase (EC 3.1.3.1), phosphodiesterase (EC 3.1.4.1) and β-N-acetylhexosaminidase (EC 3.2.1.52) were determined using the corresponding compounds with fluorescent 4-methylumbelliferone based on Marx et al. (2001), modified by Poll et al. (2006). The substrates were obtained all from Sigma–Aldrich (St. Louis, USA), except for the phosphodiesterase substrate, which was obtained from Carbosynth (Compton, UK).

For the analyses, 1 g of soil was ultra-sonicated with 50 J s^{-1} for 120 s in 50 ml of deionised H_2O. Aliquots of 50 µl of soil suspension, 50 µl buffer (0.1 M MES-buffer, pH 6.1) and 100 µl MUF-4-methylumbelliferyl-substrate dissolved in the buffer

were pipetted on microplates and incubated at 30°C. For alkaline phosphomonoesterase a modified universal buffer (pH 11) was used (Schinner et al. 1993). The increase in fluorescence over time (slope) was measured in five 30-min intervals over 180 min at 360/460 nm on a Microplate Fluorescence Reader (FLX 800, Bio-Tek Instruments, Winooski, USA) and fluorescence calculated in nmol substrate g dry soil^{-1} h^{-1} using a sample-specific standard curve with 4-methylumbelliferone added to the soil suspension.

Phospholipid and neutral lipid fatty acids

The structure of the soil microbial community was characterised by the extraction and analysis of specific phospholipid fatty acids (PLFA) (Frostegård et al. 1993, modified according to Kramer et al. 2013). Fatty acids were extracted from 2 g soil (Bardgett et al. 1996), based on the method of Bligh and Dyer (1959) and modified by White et al. (1979). Fatty acid methyl-esters were stored at -20ºC until identification by chromatographic retention time and comparison with a standard mixture of qualitatively defined fatty acid methyl-esters ranging from C11 to C20 (Sigma Aldrich, Darmstadt, Germany). Specific biomarker fatty acids allow the quantification of different microbial groups (Ruess and Chamberlain 2010; Willers et al. 2015). The PLFAs i15:0, a15:0, i16:0, and i17:0 were used as biomarkers for Gram-positive, cy17:0 and cy19:0 for Gram-negative bacteria. The sum of these fatty acids, together with 16:1ω7 and 15:0 can be used as general bacterial biomarkers. The PLFA 18:2ω6,9 was considered as biomarker for fungi (Frostegård and Bååth 1996). The sum of the bacterial and fungal markers, together with the general microbial PLFA 16:1ω5, were used as a proxy for microbial biomass.

DNA extraction

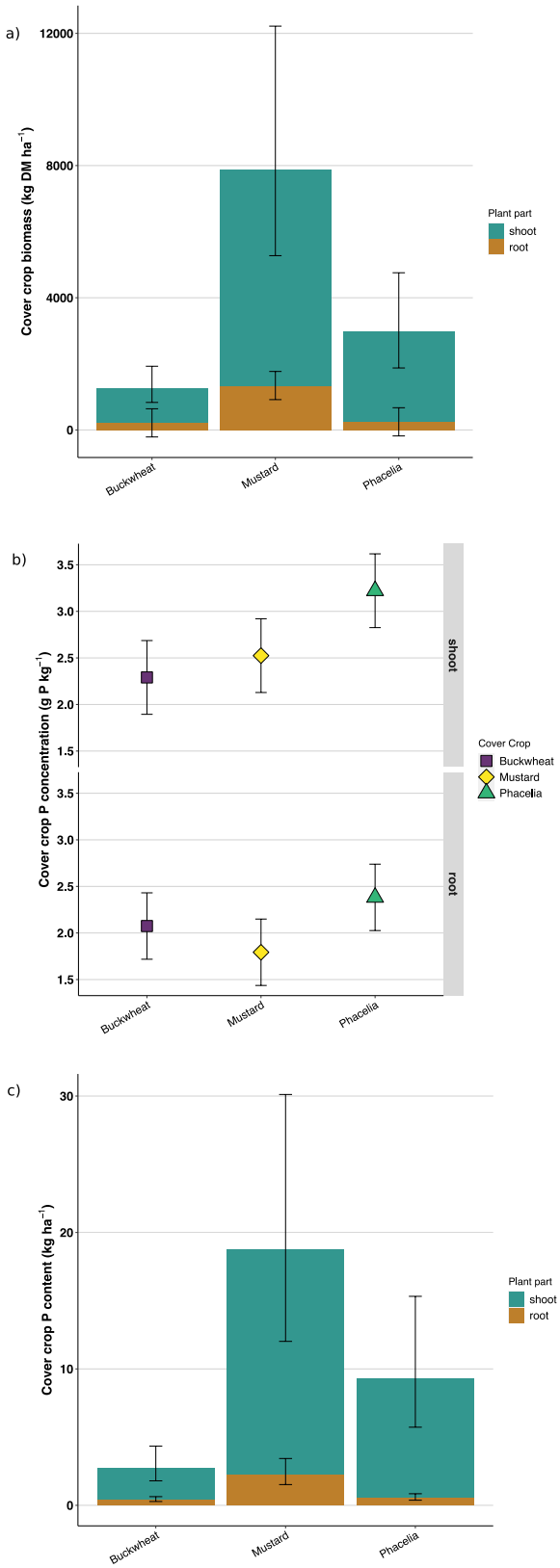
DNA was isolated from 380–400 mg rhizosheath and bulk soil samples using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Irvine, USA) and the Thermo Savant FastPrep® 120 Cell Disrupter (Thermo Scientific, Waltham, USA) according to the manufacturer's instruction. An additional washing step with 0.5 ml 5.5 M guanidine thiocyanate (Sigma-Aldrich, St. Louis, USA) was added to reduce soil contaminants. DNA quantity and quality was assessed using the NanoDrop™ 2000 spectrophotometer (Thermo Scientific, Waltham, USA). The isolated DNA was stored at − 20 °C until further analysis. Additionally, a negative control of the extraction procedure was performed without soil.

Quantitative real-time PCR (qPCR)

Bacterial 16S rRNA genes were targeted using primer pairs 341F (5'–CCT ACG GGA GGC AGC AG–3') and 515R (5'–ATT ACC GCG GCT GCT GGC A–3') (López-Gutiérrez et al. 2004) For the alkaline phosphomonoesterase gene (*phoD*) the primers *phoD*-FW (5'–TGT TCC ACC TGG GCG AYW MIA THT AYG–3') and *phoD*-RW (5'–CGT TCG CGA CCT CGT GRT CRT CCC A–3') (Bergkemper et al. 2016) were used. The bacterial 16S rRNA gene was quantified with Power SYBR™ Green PCR Master Mix using the 7500 Fast Real-Time PCR System (software version 2.3; Applied Biosystems) with a standard sequence from *Verrucomicrobium spinosum* (DSMZ 4136) according to protocols given in detail in Ditterich et al. (2013).

The qPCR for the *phoD* gene was performed in a reaction volume of 15 µl with 10 pmol·µl^{-1} of each primer, 2.5% (*v/v*) T4 Gene 32 Protein, and 5 ng DNA. The thermal profile was optimised to following conditions: 10 min at 95 °C, 5 cycles: [15 s at 95 °C, 30 s at 65 °C (-1 °C per cycle), 45 s at 72 °C], 40 cycles: [15 s at 95 °C, 30 s at 60 °C, 45 s at 72 °C, 30 s at 81 °C (measurement of fluorescence)]. The standard sequence for *phoD* originates from *Bradyrhizobium japonicum*. A PCR amplicon was obtained with the primers *phoD*-FW and *phoD*-RW using genomic DNA from the host strain prior to ligation into the vector pCR®-Blunt and cloning in *E. coli* competent cells. The purified plasmid was transformed into *E. coli* JM109 (Promega, Madison, USA) to obtain the final strain for standard preparation. The insert sequence was confirmed by Sanger-sequencing (GATC Biotech, Ebersberg, Germany). A tenfold serial dilution of the standard, ranging from 10^1–10^8 copies·µl^{-1}, was used for quantification. Amplification efficiency was accepted when exceeding 85%.

Fig. 2 Cover crop shoot and root parameters: a) plant biomass; b) phosphorus (P) concentration and c) plant P content. Displayed are the estimated marginal means of the four field replicates; error bars indicate the modelled 95% CI. The underlying data is provided in Supplementary Material 1, the structure of the fitted models and the F-tests in Supplementary Material 2 and the complete R code in Supplementary Material 3



Statistical analyses

To account for the complete block design with sampling date and soil compartment (rhizosheath vs bulk) as repeated measurements, we used linear mixed models with *block* and the interaction of *cover crop treatment, soil compartment* and date as fixed effects, and *block x soil compartment* and *block x date* as random effects (Piepho et al. 2003). The models were fitted using the package *lme4 v1.1–26* (Bates et al. 2015), in *R v3.5.0* (R-Core Team 2013) and *R-Studio v1.1.453* (RStudio 2013) and reduced by elimination of the random effects with a standard deviation of 0. The residuals were checked using Q-Q-plots and histograms (Schützenmeister et al. 2012; Kozak and Piepho 2018) and log or square root transformation was applied where appropriate. The complete R code along with the structure of the fitted models and the F-tests is provided in Supplementary Material 2 and 3. The following packages were used in the analyses: *here v1.0.1* (Müller 2020), *readxl v1.3.1* (Wickham and Bryan 2018), writexl v1.3.1 (Ooms 2020), *plyr v1.8.6* (Wickham 2011), *kableExtra v1.3.4* (Zhu 2021), *stringi v1.5.3* (Gagolewski 2018), *tidyverse v1.3.0* (Wickham et al. 2019), *cowplot v1.1.1* (Wilke 2017) as well as *RColorBrewer v1.1–2* (Neuwirth 2014) and *viridis v0.5.1* (Garnier 2018), *pbkrtest v0.5.1* (Halekoh and Højsgaard 2014) and *LmerTest 3.1–3* (Kuznetsova et al. 2017). The figures were produced with estimated means using *emmeans v1.5.4* (Lenth 2018) and *multcomp v1.4–16* (Hothorn et al. 2008).

Results

Crop biomass and nutrient content

Mustard produced by far the most biomass of any cover crop (6500 and 1300 kg ha^{-1} shoots and roots, respectively) and, with a P concentration of

2.5 and 1.8 g kg^{-1} in shoots and roots, respectively, cycling around 18 kg P ha^{-1} through its total biomass (Figs. 2a-c). Phacelia produced less biomass, but exhibited a higher P concentration in its shoots. Buckwheat produced the smallest amount of biomass of the three cover crops and had the lowest shoot P concentration, resulting in only 2.7 kg P ha^{-1} cycled through the plant biomass.

The P concentration of harvested soybean grains was not significantly changed and tended to slightly decrease by the cover crops and there were no differences among the tested cover crop species (Fig. S4).

Soil P pools.

Organic and inorganic P pools were assessed in the rhizosheath of the cover crops and in the fallow control in November 2016. For the interpretation of the effects of cover crops on soil P turnover, it is necessary to outline the soil P status. Generally, total P ranged from 922 to 1384 mg kg^{-1} soil (Fig. 3a). Organic P prevailed, with P_i accounting for only around 25% of the total P concentration. Between 174–328 mg P kg^{-1} soil (representing around 30% of the P_{org}) could be mineralised by added enzymes. Of the added enzymes, phytase released the greatest amount of phosphate, more than the sum of the phosphomonoesterase- and phosphodiesterase-labile pools (Fig. 3b).

The enzyme-labile P_{org} pools tended to be higher in the rhizosheath of cover crops compared with the fallow control (Fig. 3b). This was most evident for total enzymatically-available P_{org} and its components monoesterase-labile P_{org}, and diesterase-labile P_{org}. The rhizosheath P pools showed no differences among the tested cover crop species.

In general, P_{resin}, representing the water-soluble readily-available P pool, showed no consistent shift in the cover crop rhizosheath in November, but was affected by the plant species(Fig. S5, Supplementary Table S6). In June of the following year, we detected a strong positive effect of the growing soybean crop on P_{resin} in the rhizosheath. However, despite overall slightly higher values in the plots where cover crops had been grown over the winter, under soybean there were no differences among the cover crop species.

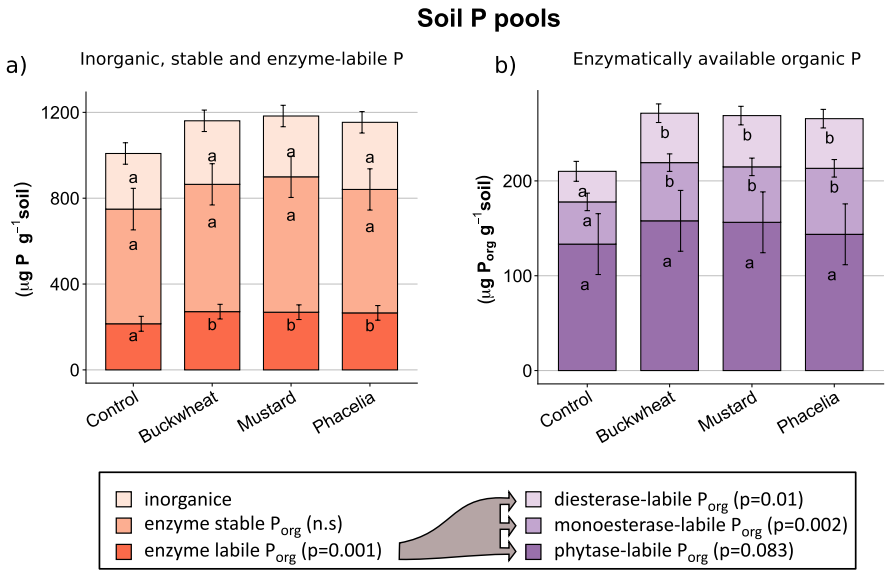


Fig. 3 Soil phosphorus (P) pools in rhizosheath soil of buckwheat, mustard and phacelia as cover crops and bulk soil of the fallow control: a) inorganic, enzyme-stable organic P (P_{org}) and enzyme-labile P_{org} in NaOH-EDTA soil extracts; b) detailed characterisation of the enzyme-available P_{org} available for phosphodiesterase, phosphomonoesterase and fungal phytase [µg P g^{-1} soil). The enzyme addition assay was conducted with rhizosheath samples of the cover crops and bulk soil of the fallow control in November 2016. The bars represent the estimated marginal means of the four field replicates, the error bars the 95% CI. Letters indicate significant differences by Tukey HSD. In the legend, the p-value for the main effect of the cover crop treatment is given (n.s. = not significant). The underlying data is provided in Supplementary Material 1, the structure of the fitted models and the F-tests in Supplementary Material 2 and the complete R code in Supplementary Material 3

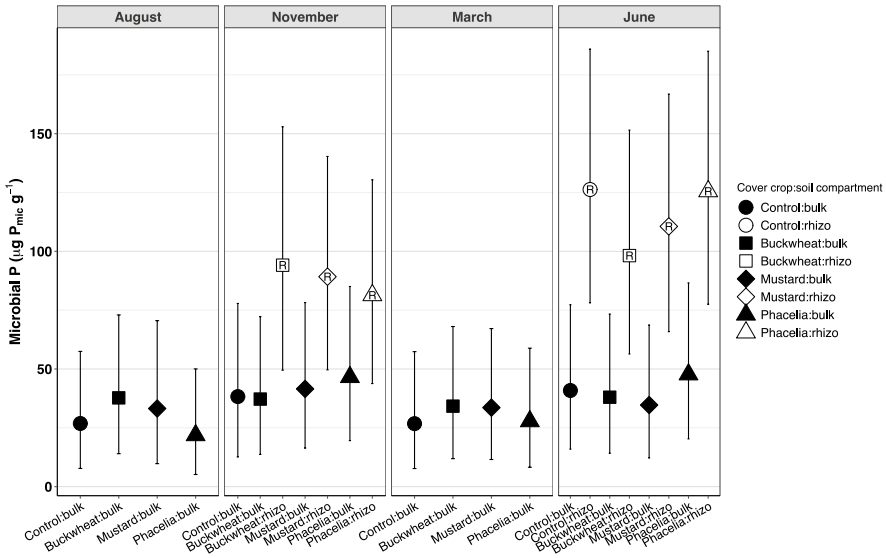


Fig. 4 Microbial biomass phosphorus (P) in µg P g^{-1} bulk (black) and rhizosheath (white) soil of the cover crop treatments over the course of the experiment. Displayed are the estimated marginal means of the four field replicates; error bars indicate the modelled 95% CI. The underlying data is provided in Supplementary Material 1, the structure of the fitted models and the F-tests in Supplementary Material 2 and the complete R code in Supplementary Material 3

The recovery of an added phosphate spike increased in the rhizosheath of cover crops and decreased in the rhizosheath of soybean (Fig. S7, Supplementary Table S6) compared to bulk soil, but the variability was generally high.

Microbial P was increased in the rhizosheath of cover crops compared with that in the bulk soil, but we detected no differences among the plant species (Fig. 4, Supplementary Table S6). We also found large increases of microbial P in the rhizosheath compared with that in bulk soil in June under soybean.

Microbial community structure

The abundance of PLFA biomarkers for Gram-positive and Gram-negative bacteria was enhanced in the rhizosheath of cover crops in November (Figs. 5a + b, Supplementary Table S6) compared with that in the surrounding bulk soil or the fallow control. The different cover crops had apparently little influence on the bacterial abundance in the rhizosheath and we found no effect in the bulk soil. In June under soybean, bacterial PLFA were also increased in the rhizosheath, but not in the bulk soil. The abundance of fungal PLFA was markedly increased in the rhizosheath of the cover crops in descending order of buckwheat > phacelia > mustard (Fig. 5c). In the phacelia-cropped plots, this increase was also found in the bulk soil in November.

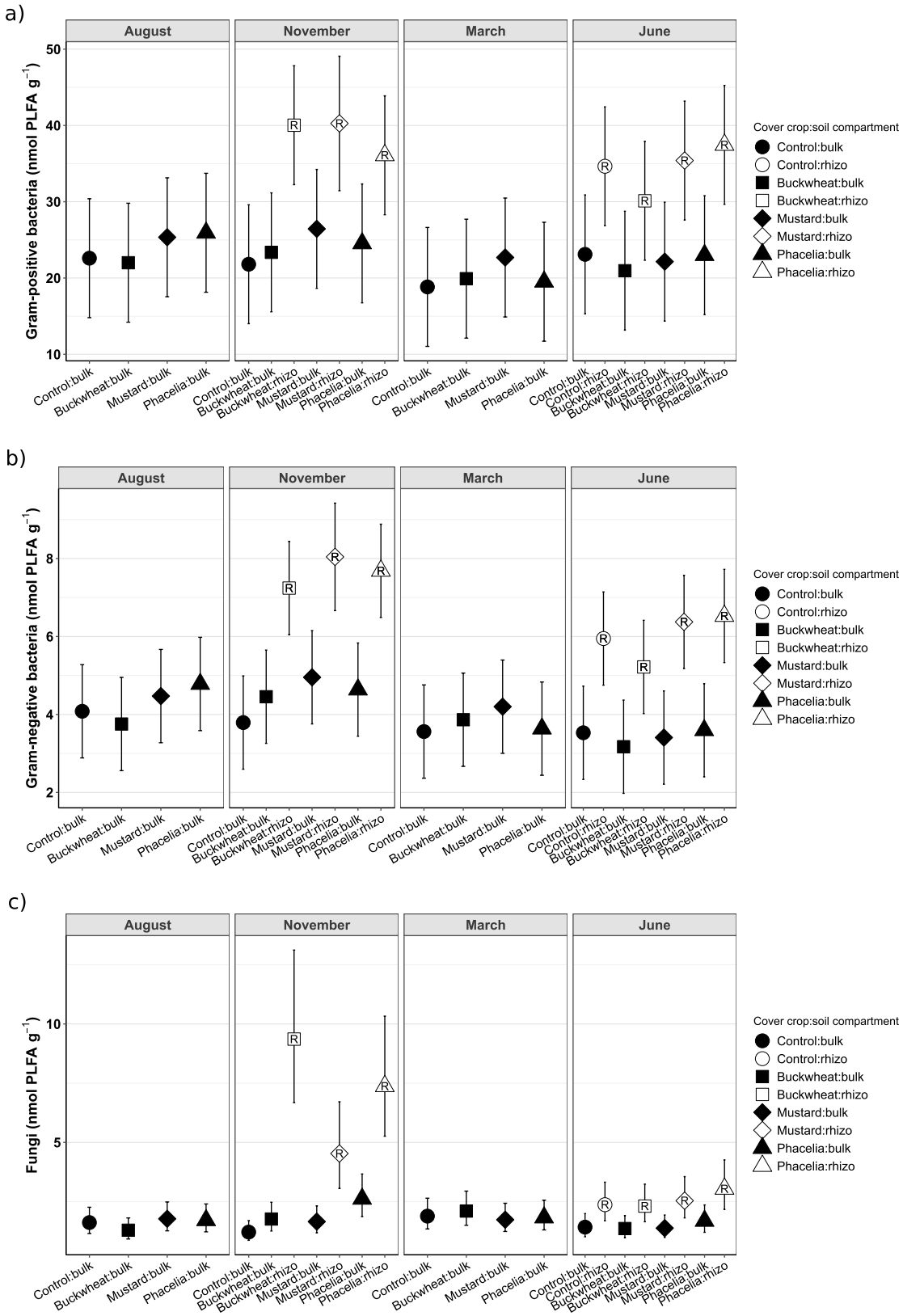
In the soybean rhizosheath, fungi were increased relative to the surrounding bulk soil. Under soybean, fungal biomass tended to be higher in the plots where formerly phacelia had been grown, but fungal abundance in the other plots had returned mostly to background levels.

The abundance of bacterial 16S rRNA genes per gram soil and the *phoD* gene, coding for alkaline phosphomonoesterase, assessed in November, were more abundant in the rhizosheath of the cover crops than in the bulk soil (Figs S8 and S9).

Microbial activity

Potential enzyme activities were higher in the rhizosheath of cover crops than in the bulk soil (Figs. 6a-d, Supplementary Table S10). The cover crops showed different activities of β-N-acetylhexosaminidase and acid and alkaline phosphomonoesterase in the following order: buckwheat > mustard > phacelia, but this trend was not significant for the the P-cycling enzymes. A positive rhizosheath effect was also found under soybean. The legacy effects of the cover crops were not straightforward, with the enzyme activities generally in the order phacelia > mustard = control > buckwheat.

The specific enzyme activity per P_{mic} of akaline phosphomonoesterase and phosphodiesterase (Figs S11b + c) was lower in the rhizosheath than in the bulk soil, while the specific acid


a)
August
November
March
June
Gram-positive bacteria (nmol PLFA g^{-1})
50
40
30
20
10
R
Control:bulk
Buckwheat:bulk
Mustard:bulk
Phacelia:bulk
Control:bulk
Buckwheat:bulk
Buckwheat:rhizo
Mustard:bulk
Mustard:rhizo
Phacelia:bulk
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Control:bulk
Buckwheat:bulk
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Control:rhizo
Buckwheat:bulk
Buckwheat:rhizo
Mustard:bulk
Mustard:rhizo
Phacelia:bulk
Phacelia:rhizo
Cover crop:soil compartment
Control:bulk
Control:rhizo
Buckwheat:bulk
Buckwheat:rhizo
Mustard:bulk
Mustard:rhizo
Phacelia:bulk
Phacelia:rhizo
b)
August
November
March
June
Gram-negative bacteria (nmol PLFA g^{-1})
8
6
4
2
R
Control:bulk
Buckwheat:bulk
Mustard:bulk
Phacelia:bulk
Control:bulk
Buckwheat:bulk
Buckwheat:rhizo
Mustard:bulk
Mustard:rhizo
Phacelia:bulk
Phacelia:rhizo
Control:bulk
Buckwheat:bulk
Mustard:bulk
Phacelia:bulk
Control:bulk
Control:rhizo
Buckwheat:bulk
Buckwheat:rhizo
Mustard:bulk
Mustard:rhizo
Phacelia:bulk
Phacelia:rhizo
Cover crop:soil compartment
Control:bulk
Control:rhizo
Buckwheat:bulk
Buckwheat:rhizo
Mustard:bulk
Mustard:rhizo
Phacelia:bulk
Phacelia:rhizo
c)
August
November
March
June
Fungi (nmol PLFA g^{-1})
10
5
R
Control:bulk
Buckwheat:bulk
Mustard:bulk
Phacelia:bulk
Control:bulk
Buckwheat:bulk
Buckwheat:rhizo
Mustard:bulk
Mustard:rhizo
Phacelia:bulk
Phacelia:rhizo
Control:bulk
Buckwheat:bulk
Mustard:bulk
Phacelia:bulk
Control:bulk
Control:rhizo
Buckwheat:bulk
Buckwheat:rhizo
Mustard:bulk
Mustard:rhizo
Phacelia:bulk
Phacelia:rhizo
Cover crop:soil compartment
Control:bulk
Control:rhizo
Buckwheat:bulk
Buckwheat:rhizo
Mustard:bulk
Mustard:rhizo
Phacelia:bulk
Phacelia:rhizo

◂**Fig. 5** Abundance of microbial groups: a) Gram-positive bacteria [PLFAs i15:0, a15:0, i16:0, and i17:0], b) Gram-negative bacteria [PLFAs cy17:0 and cy19:0], and c) saprotrophic fungi [PLFA 18:2ω6,9] in nmol of fatty acids per gram bulk (black) and rhizosheath (white) soil of the cover crop treatments over the course of the experiment. Displayed are the estimated marginal means of the four field replicates; error bars indicate the modelled 95% CI. The underlying data is provided in Supplementary Material 1, the structure of the fitted models and the F-tests in Supplementary Material 2 and the complete R code in Supplementary Material 3

phosphomonoesterase activity was not influenced by soil compartment (Fig. S11a).

Correlation of microbial community structure and function

The abundance of *phoD* copies per g soil was positively correlated with alkaline phosphomonoesterase activity (Fig. 7a, $R^2=0.39$, $p<0.001$) and the abundance of bacterial PLFAs (Fig. 7b, $R^2=0.42$, $p<0.001$). Alkaline phosphomonoesterase activity was positively correlated with bacterial PLFAs (Fig. 7c, $R^2=0.29$, $p<0.001$). The abundance of *phoD* was positively correlated with the abundance of 16S rRNA under mustard and buckwheat, but not under phacelia or in the control (Fig. 7d, $p_{cover\ crop}=0.046$). The β-N-acetyl-hexosaminidase activity was strongly correlated with fungal abundance in the rhizosheath, but not at all in the bulk soil (Fig. S12, $R^2=0.6$ and 0.0003, $p<0.001$ and 0.9, respectively).

The potential activities of acid phosphomonoesterase and phosphodiesterase were positively correlated with their corresponding labile P_{org} pools (Figs. 8a+c), while alkaline phosphomonoesterase activity showed no significant correlation with P_{org} available for added phosphomonoesterase (Fig. 8b).

Discussion

In this study, we compared soil microbial properties of three cover crops and their effects on soybean regarding soil P pools and P-cycling potential. In the rhizosheath of the cover crops, we observed an increased abundance of enzyme-labile P_{org}, as well as microbial abundance (PLFAs, P_{mic}, 16S rRNA, *phoD*) and enzyme activity relative to that in the bulk soil. Differences among the cover crop species were limited to the abundance of Gram-negative bacteria and fungi, as well as N-acetylhexosaminidase activity. Fungal abundance was correlated with the activities of phosphatases, which likely played an important role in the cycling of P_{org}. We observed only a little influence of cover crops on bulk soil or the subsequent soybean crop.

Soil P pools in the cover crop rhizosheath

The characterisation of soil P_{org} pools as a potential pool for plant nutrition was one of the principal objectives of this study. The amount of total P, and particularly $P_{org,}$ in the field, was rather high compared with the values reported in a review by Harrison (1987), despite the absence of P fertilization for over 20 years. This may be attributed to soil type, stratification and fertilisation in previous times. Considering their proportion in the P pools, organic P forms likely play an important role in soil P dynamics at this site which might also be the result of the long-term no-till management (Tiecher et al. 2012). This is reflected by the concentration of enzyme-labile P_{org}, quantified in the enzyme addition assay, which was high in comparison with the values found by Jarosch et al. (2015). The large enzyme-labile P_{org} pool indicates a high potential for soil microorganisms to have access to this fraction. The large concentration of P_{org} was responsible for these high absolute pool sizes, as the proportion of enzymatically available P_{org} of the total P_{org} was similar to that in other arable soils (Jarosch et al. 2015). In summary, the soil contained little P_i, and the P_{org} pool was remarkably large with a typical proportion of mineralisable P_{org}.

The quantity of enzyme-labile P_{org} in the rhizosheath of the cover crops was increased by about 25% compared with fallow bulk soil, driven mainly by the increases in monoesterase- and diesterase-labile P_{org} pools. The P pools were the same among the cover crop species. In November, when the sampling was carried out, buckwheat had already been killed by frost several nights before, while mustard and phacelia were reaching the end of their growing period. It is possible that rhizodeposits or dead roots contributed to the enzyme-labile P_{org} fraction. However, since we did not find a plant-specific effect, we expect that this effect was small.

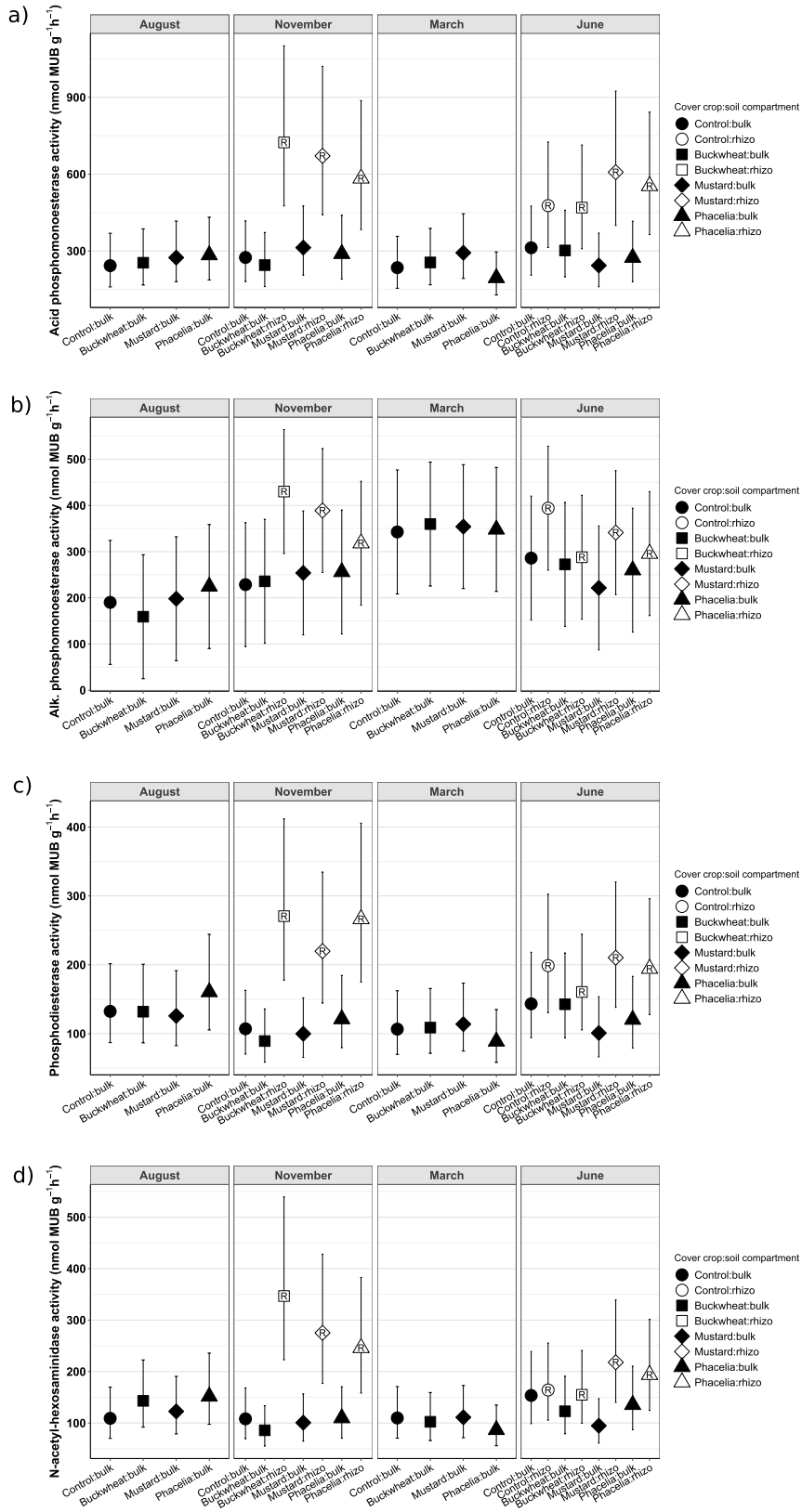

Fig. 6 Potential activities of extracellular enzymes: a) acid phosphomonoesterase; b) alkaline phosphomonoesterase; c) phosphodiesterase and d) β-N-acetyl-hexosaminidase in nmol (MUB = fluorescent methylumbelliferone) product per gram dry soil per hour in bulk (black) and rhizosheath (white) soil of the cover crop treatments over the course of the experiment. Displayed are the estimated marginal means of the four field replicates; error bars show the modelled 95% CI. The underlying data is provided in Supplementary Material 1, the structure of the fitted models and the F-tests in Supplementary Material 2 and the complete R code in Supplementary Material 3

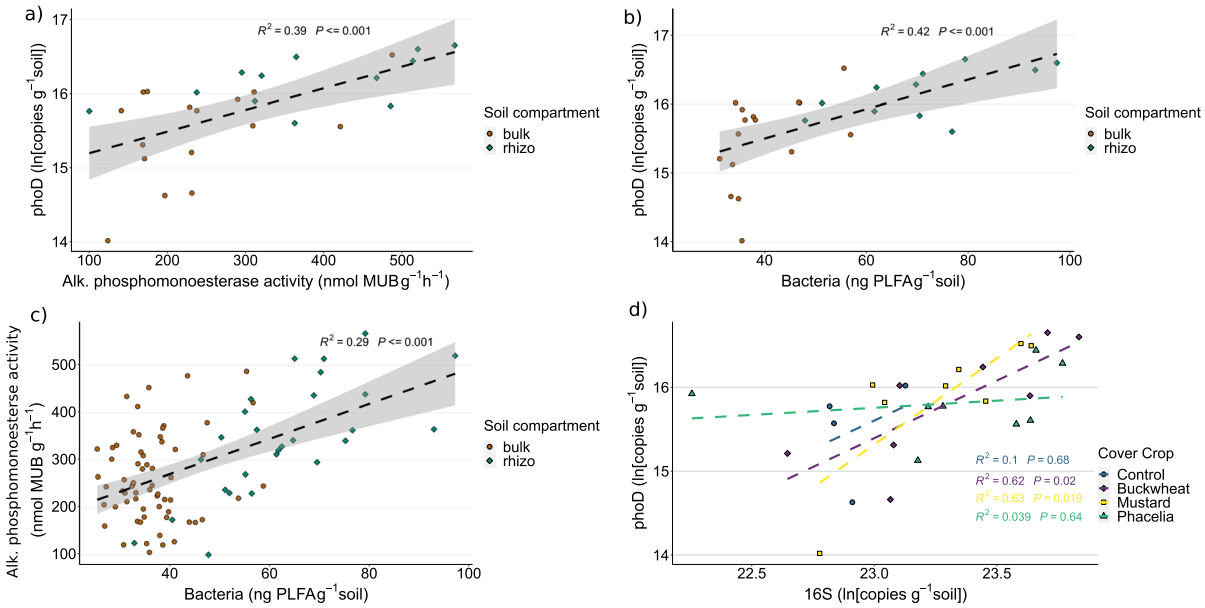

Fig. 7 Relation of the measured potential alkaline phosphomonoesterase activity with phoD, coding for alkaline phosphomonoesterase (a); bacterial PLFA with phoD (b); bacterial PLFAs with alkaline phosphomonoesterase activity (c); and abundance of the bacterial gene phoD with the abundance of bacterial 16S (d). MUB = Methylumbelliferone, corresponding to product of hidrolysis. Figure (c) has more data points than the other figures, because enzyme activity and PLFA were assessed at all sampling dates, while phoD abundance was quantified only in November. The underlying data is provided in Supplementary Material 1, the structure of the fitted models and the F-tests in Supplementary Material 2 and the complete R code in Supplementary Material 3

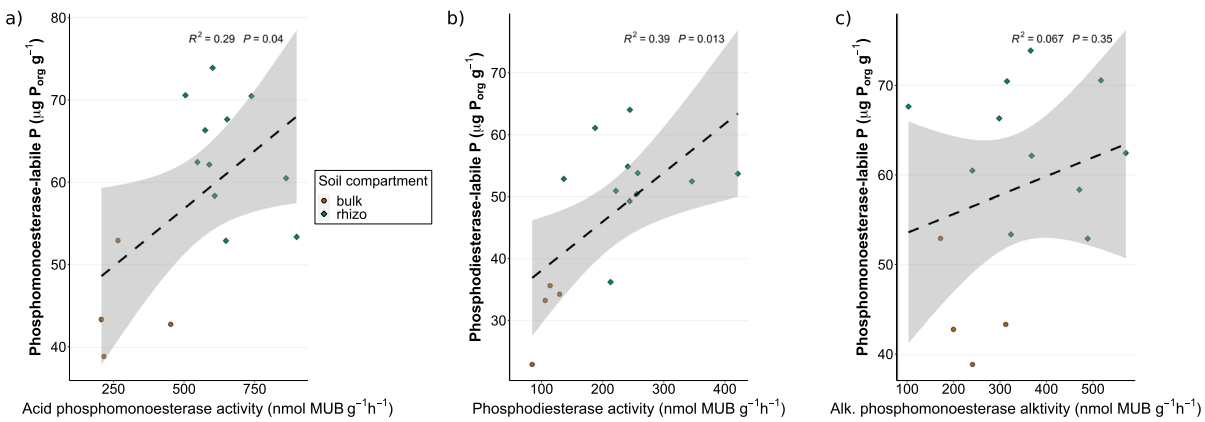

Fig. 8 Relation of measured potential enzyme activities of a) acid phosphomonoesterase, b) alkaline phosphomonoesterase and c) phosphodiesterase the corresponding enzyme-labile organic phosphorus (P) (amount P_{org} mineralised by the addition of phosphomonoesterase or phosphodiesterase in the enzyme addition assay). The underlying data is provided in Supplementary Material 1, the structure of the fitted models and the F-tests in Supplementary Material 2 and the complete R code in Supplementary Material 3

The prediction of a rhizosheath effect on the depletion/accumulation of P in low-P soils is not trivial (Hinsinger 2001), and much less so for the abundance of mineralisable P_{org}. The rhizosheath with its higher enzyme activity (and mineralisation rate of P_{org}) might have made us expect a lower abundance of

enzyme-labile P_{org}. On the other hand, it is possible that a substantial amount of the detected enzyme-labile P_{org} was derived from the necromass of soil microorganisms. The addition of C sources to soil may increase organic P forms, even without addition of inorganic P (Bünemann et al. 2008) and the use of rhizodeposits as C sources by microorganisms can be expected to follow similar mechanisms (Aerts et al. 1992). The detected increases of P_{mic} support this, but are not unequivocal proof, because the method we used for quantification detects P, and not specifically P_{org} in the microbial biomass (Kouno et al. 1995).

The pools of enzyme-labile P_{org} were positively correlated with their corresponding enzymes in the case of acid phosphomonoesterases and phosphodiesterase, but not for alkaline phosphomonoesterase. In other studies, phosphodiesterase activity correlated better with the availability of its substrate (Jarosch et al. 2019; Hallama et al. 2021) than acid phosphomonoesterase, while alkaline phosphomonoesterase has not yet been compared with the phosphomonoesterase-available pool. In accordance with this, Spohn and Kuzyakov (2013) concluded that alkaline phosphomonoesterase is not related to rhizodeposits. One reason for these results might be that microsite conditions around roots with exudation of carboxylates and protons could decouple the alkaline phosphomonoesterase activity from the availability of its substrate.

The microbial community structure and functional potential in the rhizosheath of cover crops

The results of P_{mic}, microbial PLFAs and 16S rRNA show that the microbial abundance in the rhizosheath of cover crops was increased by a factor of 2.2, 1.9 and 1.7 respectively, compared with values of the bulk soil. This rhizosheath effect corresponds to most other results of the assessed microbial properties, i.e. enzyme activity, and we suggest that microbial P cycling was responsible for the increased availability of the organic P pools.

Fungi seemed to be promoted most by cover crops, both in the rhizosheath and in the bulk soil, resulting in a persistent shift of the microbial community structure. Overall, the increase of fungal biomass in the rhizosheath of cover crops followed the order buckwheat > phacelia > mustard. Soil fungi reportedly respond to cover cropping and are sensitive to the plant species grown (Benitez et al. 2016). In our experiment, for phacelia the increase of fungal biomass could even be detected 30 weeks later under the main soybean crop. This prominent effect on saprotrophic fungi is likely connected with the particular capacities of the members of this kingdom. Their hyphal network allows fungi to connect islands of available nutrients (Ritz 1995) and water (Guhr et al. 2015), as well as enhance internal recycling and relocation of nutrients. These abilities of fungi are especially pronounced in heterogeneous soils such as under long-term no-till management (Young and Ritz 2000). The general conditions of the studied field harboured a potential for fungal growth that materialised in the rhizosheath of cover crops with the input of easily-available rhizodeposits, leading to a large increase in fungal biomass and turnover. An observed enhancement of fungal abundance with cover crops was also found in other studies (Benitez et al. 2016) and is especially interesting in view of a potential for increased soil C storage (Six et al. 2006) and other ecosystem functions (Frąc et al. 2018). Our results suggest that the trend towards bacteria-dominated soil ecosystems in more conventional agroecosystems (Frey et al. 1999) can be reversed with the use of appropriate agricultural management techniques (e.g., no-till and cover cropping).

The increases in fungal abundance involve an enhanced turnover of their biomass. Fungi are the main producers of N-acetylhexosaminidase in soils, using this enzyme for the internal recycling of the chitin contained in their cell walls. The large increase in the rhizosheath indicates a fast turnover and quick metabolism of fungal hyphae in this soil compartment (Staddon et al. 2003), probably mainly by fungi (Miller et al. 1998). During the turnover of microbial biomass, the contained nutrients are released into the soil solution and can become temporarily available for plants (Bünemann 2015).

It is often assumed that plants shape their rhizobiome to a certain extent and that this maximises benefits in terms of ecosystem function, and there is indeed evidence to support this contention (Sasse et al. 2018). However, the present results support the notion that the observed increase in 16 involves a generally enhanced microbial abundance, as we did not find a specific enrichment of specific microbial functions (i.e. potential enzyme activity or *phoD* abundance per microbial biomass), despite having used

phylogenetically very different plant species. The combined assessment of bacterial *phoD*, corresponding to the genetic potential for the production of alkaline phosphomonoesterase, and bacterial 16S rRNA, together with enzyme activities and PLFA data allow us to examine the relation among these variables. We had expected a specific enrichment of a *phoD*–harbouring population of bacteria in the rhizosheath (i.e. more *phoD* copies per bacterial 16S rRNA copies) (Figs. 7b and d), an increased expression of the gene (i.e. more alkaline phosphomonoesterase activity per *phoD*) (Fig. 7a), or an increased specific enzyme activity per unit of microbial abundance (Figs. 7c and S11b), as plants would benefit from the increased mineralisation potential. However, there was no effect of soil compartment on the correlation between *phoD* and bacterial PLFA or *phoD* and alkaline phosphomonoesterase. This suggests a general effect of an increased bacterial abundance being responsible for the observed enzyme activity in the rhizosheath. However, the relation could still be more complex. It needs to be borne in mind that *phoD* is quite ubiquitous among different microbial groups (Bergkemper et al. 2016) and our primers did not target fungal or archaeal *phoD*. Also, there are other genes that code for alkaline phosphomonoesterases, such as *phoX* (Ragot et al. 2017). Although the rhizosheath apparently had little effects on specific *phoD* gene abundance or expression, the observation that cover crop species affect the concentration of *phoD* per bacterial 16S rRNA (Fig. 7d) deserves further attention in future studies.

The interpretation of the results of the *phoD* gene is supported by the specific activity of phosphatases (the enzyme activity per unit of microbial biomass, here P_{mic}). Microbial activity, as well as microbial abundance was higher in the rhizosheath. The specific enzyme activities per μg P_{mic} of alkaline phosphomonoesterase were lower in the rhizosheath than in bulk soil (Fig S11b). Lower specific activity in the rhizosheath following the general increase of the microbial biomass makes sense in that not all microbes that benefit from the availability of rhizodeposits contribute equally to enzyme production. Notably, there were no significant rhizosheath effects for the specific activity of acid phosphomonoesterase, possibly because the plant roots themselves act as a substantial source of this enzyme (Tadano and Sakai 1991).

Cover crop roots and extension of the rhizosheath

Cover crops improve P availability through the cycling of P through their biomass (*biomass pathway*), the enhancement of the soil microbial community (*microbial pathway*) and the mining of sparingly-available P forms (*biochemical modification pathway*). The *plant biomass P pathway* is quite easily evaluated by measuring the P content of the cover crop biomass, at least when C:P and mineralisation rates are favourable (Damon et al. 2014). High plant yields would provide the greatest benefit, as cover crop biomass varies more among plant species than P concentration.

When it comes to soil-related processes (i.e. utilisation of soil P pools), the issue becomes more complex. Regarding the soil *microbial pathway*, the P contained in the microbial biomass constitutes an important pool for plant uptake (He et al. 1997), but also microbial activity and P_{org} availability are factors to consider. Even when only quantifying pool sizes, calculations of kg per ha values are hampered by the large differences between rhizosheath and bulk soil. Without an estimation of the specific rhizosheath volume and more information about its compartments, as can be obtained, e.g., with X-ray tomography (Vetterlein et al. 2020), it is not possible to quantitatively compare the different pathways of the potential cover crop-derived P benefit for the main crop under field conditions.

Although buckwheat might have had a notable effect on microbial properties and P pools in its rhizosheath, the plants had a small root biomass and, consequently, the proportion of rhizosheath volume in relation to the total bulk soil was very small in comparison with that of the other cover crops. This could explain the trend of a lower microbial abundance and activity after buckwheat compared with those after phacelia and mustard. Therefore, to assess the effect of cover crops on the following main crop, not only the magnitude of change in their rhizosheath needs to be considered, but also the size of the rhizosheath (Nannipieri et al. 2008). In addition to being affected by root biomass, rhizosheath volume depends on root architecture (root length density) and the distribution of roots (Honvault et al. 2020). When exclusively considering root morphology, mustard's rhizosheath might be underestimated in terms of rhizosphere-driven

changes on a field soil, because of its abundant root hairs and release of root exudates, which is common for Brassicaceae (Marschener 1998; Dechassa et al. 2003) and affect the size of the rhizosheath (Ndour et al. 2020; Burak et al. 2021). Moreover, their large shoot and root biomass and substantial rhizodeposits (Hunter et al. 2014) might outweigh their "unfavourable" root architecture. It may be time to revisit the widespread conception that Brassicaceae do not interact strongly with the microbial community, an idea that may be biased by their non-mycorrhizal nature.

The present matters: soybean roots dominate the soil, rather than preceding cover crops

The changes observed in the cover crop rhizosheath were rather transient and did not carry over to the main soybean crop, with the notable exception of fungal abundance. There are reports of cover-crop-induced changes of the microbial community in the main crop rhizosheath using molecular methods (Maul et al. 2014; Ortega et al. 2021). However, in the present experiment the microbial properties in the soybean rhizosheath were dominated by the growing soybean roots, and to a lesser extent by the winter cover crops that were growing on the plots before. The ecological concept of "hot spots and hot moments", coined by McClain et al. (2003) is useful to classify the importance of observed changes in the cover crop rhizosheath for the agroecosystem. In the present case, the magnitude and/or durability of the changes induced by the cover crop were not large enough to affect the soil ecosystem as a whole or the new hot spots around soybean roots. In grasslands, the observed mechanisms would probably be more important due to the permanent plant cover (Kandeler et al. 2006). Root turnover depends on climate, species and root diameter, with an estimate for temperate grasslands at a similar latitude as the present experimental field of around 0.4–0.6 yr^{-1} (Gill and Jackson 2000).

The effects of cover crops on soil microbes and nutrient cycling likely depend on the starting point and crop management. In soils with abundant microbial communities such as the present field with a long history of no-till management, cover crops might not enhance the microbial community further, while for biologically poorer systems (i.e. minimum vs conventional tillage) the relative gain could be greater (Balota et al. 2014). However, when comparing systems, the opposite can also be observed, with conventional tillage obtaining the greatest relative improvement (Wittwer et al. 2017).

Soybean belongs to Fabaceae, a family in which many species reduce the pH of the rhizosheath associated with N fixation (Hinsinger et al. 2003) and some release carboxylates and increase plant-available P fractions (Nuruzzaman et al. 2006). The expression of this mechanism is supported by the decreased P-sorption capacity in the rhizosheath of soybean ($P_{rec,}$ Fig. S7), associated with an increased concentration of plant-available phosphate (P_{resin}, Fig. S5). This biochemical rhizosheath modification might involve a close interaction with the microbial community, but this is not necessarily the case (Weisskopf et al. 2006; Spohn and Kuzyakov 2013).

Soybean performance

The field where the experiment was conducted was selected because of expected large effects of cover crops on main crop nutrition due to a low concentration of available P. The P concentration of the soybean grains in this experiment was in the lower range of values reported by Xie et al. (2017), but soil P availability was probably not the most important limiting factor. Agronomically, the main crop did not benefit from the preceding cover crops in terms of P concentration. This absence of a positive effect on the main crop makes it difficult to draw conclusions about the relative importance of the pathways of P benefits outlined in the introduction. In the present study, the plant biomass pathway was apparently not very important, as the considerable amount of P cycled through the mustard biomass did not affect soybean P concentration. A shorter timespan between cover crop death and sowing of the main crop might have improved the synchronisation of P release from the plant litter (Damon et al. 2014) This highlights the dependence of cover crop results on management (Wittwer et al. 2017) and site conditions (Blanco-Canqui et al. 2015), while the potential of enhanced P-transformation in agroecosystems by increasing above- and belowground biodiversity might require time to unfold (Oelmann et al. 2021). A fact that could be relevant for the (agronomic) results of this study is that the main crop was a legume and the cover crops were not. A different combiation with a (non-legume) main crop might

well have had greater benefits from the cover crops (Tonitto et al. 2006).

Conclusions

This on-farm experiment evaluated the correlation between the availability of P_{org}, the microbial community and P-cycling enzymes in the bulk and rhizosheath soil of buckwheat, mustard and phacelia as cover crops and in the following soybean crop on a soil low in plant-available P_i, but with abundant P_{org}. Our findings confirm our first hyptothesis, as cover crops greatly enhanced the amount of enzymatically-available $P_{org,}$ as well as microbial abundance and activity in their rhizosheath, showing a potential to increase the cycling of P_{org}. Our second hypothesis was not confirmed, as the fact that most microbial properties did not differ greatly among the tested cover crop species indicates that the sheer presence of a living plant was more important than the nature of the species. However, in an experiment with a longer duration, the impact of several successive cycles with the same cover crop probable would have produced a larger impact on P cycling.

The large effects of cover crop species on fungi indicate that the potentially important role of fungi in P cycling deserves more attention. This is to be understood in the context of our observation that currently in the scientific community there seems to be more attention on the development of sophisticated methods targeting bacteria, rather than fungi or other soil biota.

We found no evidence for a specific enrichment of microbes providing beneficial functions such as an overproportional increase of a *phoD*-harbouring bacterial populations or the specific enzyme activity per unit of microbial biomass. Contrary to our third hypothesis, the observed increases in microbial function in the rhizosheath of cover crops might therefore be related more to an overall increase of the microbial abundance and its turnover due to the availability of rhizodeposits than to specific shifts of the microbial community.

The observed cover crop-induced changes to bacterial abundance and activity of several P-cycling enzymes were spatially and temporally restricted and, contrary to our fourth hypothesis, soybean grain P concentration was not affected by cover cropping. The differentiation between the rhizosheath and bulk soil indicates that the rhizosheath volume (i.e. root density and architecture) needs to be taken into account when estimating potential cover crop effects. For future studies, it is recommended to consider the three dimensional heterogeneity of P cycling processes and soil microorganisms in the bulk soil and the rhizosphere.

It is important to bear in mind that the current management of the field without application of any fertilisers, but nutrient export with harvest, represents a form of P-mining that can only be sustained during a limited timespan until the sparingly-available P reserves will become exhausted. However, to decrease pressure on the limited mineable P reserves and reduce environmental hazards from the overapplication of fertilisers, it might be worth to investigate the management options to extend this period.

In summary, we confirmed that cover crops can be used to locally modify plant-available P pools, and their enhanced rhizobiome affects different functions involved in P cycling. Organic P is an important component of the cycling of terrestrial P, and should be taken more into consideration.

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Availability of data and material The underlying data is available in Supplementary Material 1 and at the public repository *Open Science Framework* (https://osf.io/yh5ra/).

Code availability The R-code used for the statistical analysis is available in Supplementary Material 3 and at the public repository *Open Science Framework* (https://osf.io/yh5ra/).

Declarations

Conflicts of interest/Competing interests Not applicable.

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