Soil microorganisms as hidden miners of phosphorus in soils under different cover crop and tillage treatments

Dissertation to obtain the doctoral degree of Agricultural Sciences

(Dr. sc. agr.)

Faculty of Agricultural Sciences

University of Hohenheim

Institute of Soil Science and Land Evaluation

submitted by

Moritz Hallama

Born in Stuttgart

2021

This thesis was accepted as a doctoral dissertation in fulfillment of the requirements for the degree “Doktor der Agrarwissenschaften” (Dr. sc. agr. / Ph.D. in Agricultural Sciences) by the Faculty of Agricultural Sciences at the University of Hohenheim, Germany on: 26.01.2022

Date of oral examination: 13.06.2022

Examination committee

Reviewer and supervisor:	Prof. Dr. Ellen Kandeler
Co-reviewer:	Prof. Dr. Yvonne Oelmann
Additional examiner and Co-supervisor:	Prof. Dr. Carola Pekrun
Chair of Committee:	Prof. Dr. Thilo Streck

This thesis was conducted at the Institute of Soil Science and Land Evaluation at the University of Hohenheim. Financial support was provided by the “Landesgraduiertenförderung” by the Faculty of Agricultural Sciences at the University of Hohenheim from January 2015 to October 2016 and from November 2016 to September 2019 by the European Union's Horizon 2020 research and innovation program under grant agreement No 677407 (SoilCare project).

Acknowledgements

It is an illusion that science could be produced by the author alone. This thesis would not have been possible without the support of many. Thanks to all of you! And to all the people that are not mentioned explicitly. You know who I mean.

First, I want to honor my supervisors, Prof. Dr. Ellen Kandeler, from the Department of Soil Biology at the University of Hohenheim, and Prof. Dr. Carola Pekrun, from the Agronomy Section, Institute of Applied Agriculture at Nuertingen-Geislingen University. Dear Ellen, I learned a lot from you. We certainly had our rough times, but also wonderful talks and walks in the mountains of Crete, where I could grasp the inspiration of true mentorship. Your knowledge of soil biological methods might surprise initially, but knowing that it was you who participated in the development of many of them gives a hint about your capacities. You certainly shaped the scientist in me. Dear Carola, you are an amazing professor and a very kind person. Your work has a much larger influence on how our farmers treat the soil than you probably are aware of. You are an absolute asset for your university. Dear Hans, although not being officialy my supervisor, you had definitely your part. But besides gratitude, I have questions to you. How do you manage to do all the things you accomplish in a day? Do you sleep at all? And how can you be simultaneously so brilliant and so hilarious?

You, dear co-authors, helped me to write this piece. Your critical comments made me improve my writing, while your warm words lifted my mood and helped me to bear the dark hours of revisions. Dear Kathy, your hawk-eye spotted my mistakes and made my writings blossom.

My colleagues at the department supported me daily with knowledge, help and community. If someone doesn't know: Every day at coffee time, at the Institute of Soil Science you can find a wonderful bunch of people around a table resolving the minor and major problems of existence. I hope you enjoyed the time with me as I certainly did with you.

At the university, it is difficult to look under a bush without stepping on a remarkable person. Maybe it's not you who make the world turn around, but at least you are a hard-working hood to figure out why it does. A special mention goes to Prof. Piepho, the best

statistician in the world (with a high probability). I hope that some day, education will be all like the magnificent, self-organized statistics seminar you inspired. Seriously. This gives me hope.

I am also fortunate having formed part of the SoilCare Project. Here I met very good scientists and feel now part of a Europe-wide network. Further, I want to thank the members of the Projekt *Konservierender Ackerbau* who achieve the difficult task of adapting the idea of conservation agriculture to the reality. I want to give a special tribute to Rolf Kern, whose advice and incessant work made an entire region green. Hopefully my work is of use in your task.

I am deeply grateful to the people that suggested me to study the topic of phosphorus and made me realize that for humanity, sustainable agricultural is not an option, but a necessity: Aksel Naerstadt, Hans Herren, Johannes Storch, Stefan v. Bonin, Jan-Hendrik Cropp, Stephan Junge, Julia Bar-Tal, and my father, Uli. I want to mention especialy my mother, Ramona, whose wise words made me continue, even when I doubted if all this effort would be of any good. My friends, whose support motivated me, and who are still out there, awaiting my return to the living.

This work, and my mere existence would not have been possible without the efforts of innumerable farmers and workers, bearing the weight of the world on their shoulders. My task is now to use my privileges wisely.

But most of all my family, who had to endure a stressed PhD-Student all this time. Marian, without your aid I never would have made it through. You stayed calm when I lost my nerves. You gave me excellent advice, because you are an admirable scientist. You gave me a hug when I was in need. *Gracias por acompañarme*, *guapa*.

I want to dedicate this thesis to my Kids, Uba and Aran. My next project is to recover the lost time with you.

Glossary

AMF: Arbuscular Mycorrhizal Fungi

C_{mic}: Microbial biomass C

EAA: Enzyme Addition Assay

LDA: Linear Discriminant Analysis

NLFA: Neutral Lipid Fatty Acids

NT: No-till/direct seeding

P_a: Plant-available phosphorus

P_{CAL}: Calcium-acetate-lactate extractable P

P_i: inorganic Phosphorus (orthophosphate)

PLFA: Phospholipid Fatty Acids

P_{mic}: microbial biomass Phosphorus

P_{org}: organic phosphorus

P_t: Soil total phosphorus

RT: Reduced/non-inversion tillage

SOM: Soil organic matter

Table of Contents

ACKNOWLEDGEMENTS ..... **III**

GLOSSARY ..... **V**

TABLE OF CONTENTS ..... **VI**

LIST OF FIGURES ..... **VIII**

LIST OF TABLES ..... **XII**

LIST OF SUPPLEMENTARY MATERIAL ..... **XIII**

1 SUMMARY **1**

2 ZUSAMMENFASSUNG **3**

3 GENERAL INTRODUCTION **6**

4 OBJECTIVES **8**

5 STUDY #1: HIDDEN MINERS – THE ROLES OF COVER CROPS AND SOIL MICROORGANISMS IN PHOSPHORUS CYCLING THROUGH AGROECOSYSTEMS **12**

5.1 Abstract 13

5.2 Introduction 13

5.3 The Meta-Analysis 29

5.4 Results of the Meta-Analysis 33

5.5 Discussion 40

5.6 Conclusions 62

5.7 Acknowledgements 65

5.8 Supplementary Material 66

6 STUDY #2: THE ROLE OF MICROBES IN THE INCREASE OF ORGANIC PHOSPHORUS AVAILABILITY IN THE RHIZOSHEATH OF COVER CROPS **68**

6.1 Abstract 69

6.2 Introduction 70

6.3 Materials and Methods 72

6.4 Results 79

6.5 Discussion 89

6.6 Conclusions 96

6.7 Acknowledgements 97
6.8 Supplementary Material 98

7 Study #3: Interactions between cover crops and soil microorganisms increase phosphorus availability in conservation agriculture 106

7.1 Abstract 107
7.2 Introduction 107
7.3 Materials and Methods 110
7.4 Results 118
7.5 Discussion 129
7.6 Conclusions 138
7.7 Acknowledgements 138
7.8 Supplementary Material 139

8 General discussion 145

8.1 How do cover crops increase the access of P to main crops? 145
8.2 How are soil microbial functions and the enzymatic availability of P_{ORG} pools under cover crops connected? 150
8.3 Agricultural management and plant-soil-microbe interactions 153

9 Conclusions and perspectives 155

10 References 160

Declaration 203

Curriculum vitae 204

List of Figures

Figure 5.1 *Strategies and mechanisms for phosphorus (P) acquisition by plants: 1) soil exploration via roots and mycorrhizal hyphae; 2) mobilization of sparingly-soluble inorganic P (P_i) and organic P (P_{org}) by exudation of H^+/OH^- and carboxylates; 3) mineralization of P_{org} by phosphatases. Plant-driven processes have solid outlines, microbial activity is shown by dotted outlines*

Figure 5.2 *Pathways of phosphorus (P) transfer and plant-microbial processes affecting P availability by cover cropping. 1) Soil P pools of varying degrees of availability are solubilized and/or mineralized and are immobilized in the microbial biomass. 2) The microbial biomass releases P into the soil solution which 3) ends up in the plant via root or mycorrhizal uptake. Cover crops may additionally possess the capacity to mine P from poorly-available P pools or to produce biochemical rhizosphere modifications to increase P availability. 4) The roots release rhizodeposits that shape the microbial community, eventually leading to increased P mining. 5) The P stored in the cover crop biomass is transferred to the main crop via cover crop residues, which are decomposed by the soil microbial community (6). The soil microbial community (i.e. mycorrhizal fungi) in the main crop phase, enhanced by the cover crops, may possess an increased capacity to mine P for the main crop (7).*

Figure 5.3 *Cover crop dry matter biomass [t ha^{-1}], shoot phosphorus (P) content [kg ha^{-1}] and concentration of P in biomass [g kg^{-1}]. The points represent the modeled median (+/- 95 % CI) of the different cover crop treatments. On the left are displayed the number of observations. The letters indicate significant differences among cover crop types with a Tukey post-hoc test ($p<0.05$). The corresponding models can be found in Supplementary Material S5.3.1*

Figure 5.4 *Change in main crop yield and shoot biomass following cover crops from different families. The points represent the modeled median (+/- 95 % CI), relative to the respective controls. On the left are displayed the number of observations. The lower-case letters indicate, for a single main crop type with a Tukey post-hoc test ($p<0.05$), significant differences among cover crop types (including the control) and the upper-case letters between cover cropping in general and the controls. The corresponding models can be found in Supplementary Material S5.3.2*

Figure 5.5 *Change in main crop yield and shoot biomass after different Fabaceae cover crop genera and species. The points represent the percentage change of the modeled median (+/- 95 % CI), relative to the respective controls. On the left are displayed the number of observations. The letters indicate significant differences among cover crop species with a Tukey post-hoc test ($p<0.05$). Species with only one or two observations were aggregated as "other Fabaceae": Anthyllis vulneraria, Tephrosia purpurea, Stylosanthes guianensis, Pueraria phaseoloides, Psophocarpus tetragonolobus, Mucuna cochinchinensis, Dolichos lablab, Cassia tora, Canavalia ensiformis, Cajanus cajan, Trifolium sp., Arachis hypogaea. The corresponding model can be found in Supplementary Material S5.3.3*

Figure 5.6 *Soil biological parameters: change in percent abundance of arbuscular mycorrhizal fungi (AMF), microbial biomass phosphorus (P) content, and phosphatase activity as well as available P after different cover crops, relative to the respective controls. On the left are displayed the number of observations. The lower-case letters indicate, for a single main crop type with a Tukey post-hoc test ($p<0.05$), significant differences among cover crop types (including the control), and the upper-case letters between mycorrhizal cover crops, nonmycorrhizal cover crops and the controls. The corresponding models can be found in Supplementary Material S5.3.5*

Figure 5.7 *Main crop yield and phosphorus (P) uptake as affected by cover cropping in soils with low and high available P (P_a). The points represent the percentage change of the modeled median (+/- 95 % CI) of the cover crop treatments relative to the controls without cover crops. On the left are displayed the number of observations. The letters indicate significant differences among groups with a Tukey post-hoc test (p<0.05). The corresponding models are presented in Supplementary Material S5.3.6*

Figure 5.8 *Effect of cover crops on arbuscular mycorrhizal fungi (AMF) in soils with different P availability. The points represent the percentage change of the modeled median (+/- 95 % CI) of the cover crop treatments relative to the controls without cover crops. On the left are displayed the number of observations. The letters indicate significant differences among groups with a Tukey post-hoc test (p<0.05). The corresponding models are presented in Supplementary Material S5.3.7*

Figure 5.9 *Radar chart summarizing the properties of the cover crop families and their effects on soil. The lines correspond to the calculated quantile moment of each data point (R code provided in Supplementary Material S5.4). Grid lines correspond to the 0, 25, 50, 75, and 100-quantiles of each variable. Asteraceae and Polygonaceae had missing data points and could not be displayed here.*

Figure 6.1 *Soil cover, sampling scheme and a view of the field experiment of Study #2.*

Figure 6.2 *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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Figure 6.3 *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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Figure 6.4 *Microbial biomass phosphorus (P) in mg 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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Figure 6.5 *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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Figure 6.6 *Potential activities of extracellular enzymes: a) acid phosphomonoesterase; b) alkaline phosphomonoesterase; c) phosphodiesterase and d) 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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Figure 6.7 *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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Fig 6.8 *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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Figure 7.1 *Climate and management of the field experiment of Study #3. Top: climate chart (left y-axis: monthly average air temperature [°C], right y-axis: cumulative monthly precipitation [mm]). Bottom: sampling (February and October 2015), soil cover and management (RT: reduced tillage). Further management details are listed in Supplementary Material S7.1*

Figure 7.2 *Soil P pools at Tachenhausen field site in 0-5 cm. a) In the left figure, the top, middle and bottom bars correspond to inorganic P (P_i), enzyme-stable organic P (P_{org}) and enzyme-labile P_{org}., respectively b) The enzyme-labile P pool can be further subdivided into P_{org} hydrolysable for phosphodiesterase, non-phytase-phosphomonoesterase and fungal phytase (bare= without cover crops, RT= reduced tillage, NT= no-till). The bars represent the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding models and F-Tests can be found in Table 1 and Supplementary Material S7.3*

Figure 7.3 *Calcium acetate lactate extractable phosphate (P_{CAL}) under the different treatments at 0-5 cm (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 %. The corresponding model and F-test can be found in Supplementary Material S7.3*

Figure 7.4 *Microbial biomass: a) microbial C measured as substrate induced respiration (SIR) [µg microbial C g^{-1} soil], b) concentration of microbial PLFA biomarkers [nmol PLFA g^{-1}] and c) microbial P [µg P g^{-1}] by treatments at 0-5 cm (bare= without cover crops, RT= reduced tillage, NT= no-till, bare= without cover crops). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding models and F-Tests can be found in Supplementary Material S7.3*

Figure 7.5 *Concentration of fatty acid biomarkers of microbial groups: a) Gram+ [PLFAs i15:0, a15:0, i16:0, and i17:0], b) Gram- bacterial [PLFAs cy17:0 and cy19:0], c) general fungal [PLFA 18:2ω6,9 and 18:3ω6,9,12], and d) arbuscular mycorrhizal biomarkers [NLFA 16:1ω5] in nmol of fatty acids per gram dry soil under the different treatments at 0-5 cm (bare= without cover crops, RT= reduced tillage, NT= no-till, bare= without cover crops). Displayed are the estimated marginal means of the three field replicates; error bars indicate the modelled 95 % CI. The corresponding models and F-Tests can be found in Supplementary Material S7.3*

Figure 7.6 *Potential activities of extracellular enzymes: a) phosphomonoesterase and b) phosphodiesterase in nmol of substrate per gram dry soil per hour under the different treatments at 0-5 cm (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding models and F-Tests can be found in Supplementary Material S7.3*

Figure 7.7 *Relation between enzymatic activity and the respective enzyme-available organic P pools for a) phosphomonoesterase and b) phosphodiesterase. The trend lines, R^2 and p-values were calculated using a simple linear model. As the relation of enzymatic activity and P_{org} pools interacted with depth and tillage as well as date and cover crops in the case of phosphomonoesterase and phosphodiesterase, respectively, the trendlines were fitted to the corresponding subsets. Coefficients and R-code can be found in Supplementary Material S7.3 and S7.4, respectively.*

Figure 7.8 *Impact of cover crops (green= cover crops, brown= fallow) and tillage (light= reduced tillage/RT, dark= no-till/NT) on microbial community structure (a, b; fatty acid biomarkers) and activity (c, d; extracellular enzyme activity and substrate use capacity), in February (left) and October (right) at 0-5 cm, grouped by treatment. The parameters of each plot are summarised to a single point using linear discriminant analysis (LDA). The ellipses represent the 95 % CI of each group. Coefficients and R-code can be found in Supplementary Material S7.3 and S7.4, respectively*

Figure 7.9 *Radar chart summarizing the effects the four treatments of the experiments (bare vs cover crops and reduced tillage vs no-till), on several soil phosphorus pools and microbial P-cycling in February at 0-5 cm. The variables represent (clockwise from the top right): Microbial abundance (Gram+ and fungal abundance); Soil P pools (fungal phytase-labile organic P (P_{org}), calcium-acetate-lactate extractable P (P_{CAL}), total Phospholipids (PLFAs), microbial biomass phosphorus; and enzyme activity (phosphodiesterase and phosphomonoesterase). Grid lines correspond to the 0, 25, 50, 75, and 100-quantiles of each variable over all dates and depths (R-code can be found in Supplementary Material S7.4)*

List of Tables

***Table 5.1**: Plant species widely used for cover cropping and their properties as described in the literature*

***Table 7.1** P-values for main effects and interactions of the fitted models of different P pools presented in Fig. 7.2. The factor levels were: cover crops (bare and cover crops), tillage (no-till and reduced tillage), date (Ferbuary and October) and depth (0-5 and 5-20 cm). The corresponding raw data can be found in Online Resource S7.2, models and full ANOVA tables in Online Resource S7.3, and the corresponding R code in Online Resource S7.4. Interactions where no significance was detected were omitted*

List of Supplementary Material

Supplementary Material S5.1 *Overview of the studies used for the meta-analysis. Available at the public repository Open Science Framework (https://osf.io/nr7km/)*

Supplementary Material S5.2 *Database of the meta-analysis. Available at the public repository Open Science Framework (https://osf.io/nr7km/)*

Supplementary Material S5.3 *Structure of the fitted models and F-Tests of the ANOVAs of the fixed effects. Available at the public repository Open Science Framework (https://osf.io/nr7km/)*

Supplementary Material S5.4 *Sample R-code of the statistical analysis. Available at the public repository Open Science Framework (https://osf.io/nr7km/*

Figure S5.5 *Change in main crop phosphorus (P) content after cover crops belonging to different families. The points represent the modeled median (+/- 95 % CI) relative to the respective controls, averaged over all main crops. On the left are displayed the number of observations. The lower-case letters indicate, for a single main crop type with a Tukey post-hoc test (p<0.05), significant differences among cover crop types (including the control), and the upper-case letters differences between cover cropping in general and the controls. The corresponding models are presented in Supplementary Material S5.3.4*

Figure S5.6 *Main crop yield and phosphorus (P) uptake as affected by cover cropping under different management regimes: noninversion tillage plus fertilization; noninversion tillage without fertilization; conventional tillage plus fertilization and conventional tillage without fertilization. Care has to be taken in the interpretation of the results of the no-till plus fertilization treatments due to the low number of observations. The points represent the percentage change of the modeled median (+/- 95 % CI) of the cover crop treatments relative to the controls without cover crops. On the left are displayed the number of observations. The letters indicate significant differences among groups with a Tukey post-hoc test (p<0.05). The corresponding models are presented in Supplementary Material S5.3.8.*

Supplementary Material S6.1 *Excel file with the full dataset. Available at the public repository Open Science Framework (https://osf.io/yh5ra/)*

Supplementary Material S6.2 *Details of the fitted models and full ANOVA tables. Available at the public repository Open Science Framework (https://osf.io/yh5ra/)*

Supplementary Material S6.3 *R-code of the statistical analysis. Available at the public repository Open Science Framework (https://osf.io/yh5ra/)*

Figure S6.4 *Phosphorus (P) concentration of harvested soybean grains in mg P kg^{-1}. 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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Figure S6.5 *P_{resin}, the concentration of resin-extractable available phosphorus (P) [µg P g^{-1} soil] 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 indicate the modelled 95% CI. The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Supplementary Material S6.6 *Overview of the p-values for main effects and interactions of the fitted models of P_{resin} (resin-extractable inorganic phosphorus (P)), P_{mic} (microbial biomass P), P_{rec} (recovery of added P spike, indicates P-sorption capacity) and abundances of fatty acid biomarkers of different microbial groups. The factor levels were: soil compartment (rhizosheath vs bulk soil), cover crop treatment (buckwheat, mustard, phacelia and fallow control), date (August and November 2016, March and June 2017). 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 R-code in Supplementary Material 3*

Figure S6.7*: Recovery of added phosphorus (P) spike in %, the inverse of soil P-sorption capacity 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 indicate the modelled 95% CI. The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Fig S6.8 *Bacterial 16S rRNA per gram soil soil in bulk (black) and rhizosheath (white) soil of the cover crop treatments in November 2016. 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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Figure S6.9 *Bacterial phoD gene abundance in copy numbers per gram dry soil in bulk (black) and rhizosheath (white) soil of the cover crop treatments in November 2016. 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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Supplementary Material S6.10 *Overview of the p-values for main effects and interactions of the fitted models of different enzyme activities presented in Fig. 6.6. The factor levels were: soil compartment (rhizosheath vs bulk soil), cover crop treatment (buckwheat, mustard, phacelia and bare fallow control), date (August and November 2016, March and June 2017). 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 R-code in Supplementary Material 3*

Figure S6.11 *Ppotential activities of extracellular phosphatase enzymes per microbial biomass phosphorus (P): a) acid phosphomonoesterase; b) alkaline phosphomonoesterase and c) phosphodiesterase in nmol of (fluorescent) substrate µg P_{mic} 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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Figure S6.12 *Relation of the abundance of fungal PLFA 18:2ω6,9 with measured potential N-acetyl-hexosaminidase (NAGase) activity. The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Supplementary Material S7.1 *Spreadsheet (.xlsx) with the agronomic management. Available at the public repository Open Science Framework (https://osf.io/m9d5c/)*

Supplementary Material S7.2 *Spreadsheet (.xlsx) with the full dataset. Available at the public repository Open Science Framework (https://osf.io/m9d5c/)*

Supplementary Material S7.3 *Document (pdf) with fitted models and results of ANOVA. Available at the public repository Open Science Framework (https://osf.io/m9d5c/)*

Supplementary Material S7.4 *R-code (.Rmd) used for statistical analysis and elaboration of figures. Available at the public repository Open Science Framework (https://osf.io/m9d5c/)*

Figure S7.5 *Organic P hydrolysed by added bacterial phytase under the different treatments (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding model and F-test can be found in Supplementary Material S7.3*

Figure S7.6 *Resin-P (P_{resin}) in soil-water-extract under the different treatments (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding model and F-test can be found in Supplementary Material S7.3*

1 Summary

Phosphorus (P) is one of the most limiting plant nutrients for agricultural production. The soil microbial community plays a key role in nutrient cycling, affecting access of roots to P, as well as mobilization and mineralization of organic P (P_{org}). This thesis aimed to better understand the potential of cover crops to enhance plant-soil-microbe interactions to improve the availability of P.

The meta-analysis of 25 published field studies allowed us in Study #1 to articulate a comprehensive framework of cover crop-derived P benefits. Following our review, the field experiments of Studies #2 and #3 added to our understanding of the underlying mechanisms driving P availability, with special emphasis on the role of microbes. The field experiments were conducted on loess-derived soils in southwestern Germany with winter cover crops and soybean as main crop in randomized complete block designs with four replicates. Study #2 was designed to evaluate the plant-soil-microbe interactions of three cover crop species (mustard, phacelia and buckwheat) in their rhizosheaths and was carried out on a field low in available P. Study #3 investigated the interactions of a cover crop mixture with tillage treatments of different intensity under conditions of an abundant availability of inorganic P in 0-5 and 5-20 cm soil depth. In the field experiments, a comprehensive set of microbial properties, including microbial abundance, community structure (phospholipid fatty acid biomarkers, PLFAs), P-cycling enzymes and microbial biomass P, was linked for the first time with the lability of P_{org} pools for enzymatic mineralisation. Additionally, in Study #2, the abundance of 16S-rRNA and *phoD*, coding for alkaline phosphomonoesterase in bacteria, were quantified using real-time qPCR, while in Study #3 the carbon-substrate use capacity of the microbial community was additionally assessed.

The used methods showed that microbial P, the activity of P-cycling enzymes and PLFAs increased under cover crops, indicating an enhanced potential for organic P cycling. Gram-positive and Gram-negative bacteria, and to a lesser extent also arbuscular mycorrhizal fungi, increased their abundance with cover crops. However, saprotrophic fungi could benefit most from the substrate input derived from cover crop roots or litter. Enzyme-stable P_{org} shifted towards pools of a greater lability in the active soil compartments (rhizosheath

and detritusphere). The effects of agricultural management, such as cover crop species choice and tillage, were detectable, but weaker compared to the effect of the presence of cover crops.

With the obtained results, the research aims of this thesis could be successfully addressed. We were able to confirm that cover crops have the potential to improve main crops' access to P. Furthermore, we presented and discussed three pathways of P benefit. In the *plant biomass pathway*, P is cycled through cover crop biomass and becomes available for the main crop upon litter decomposition. The *microbial enhancement pathway* describes how the cover crop's interaction with soil microbes increases their abundance and activity, thereby increasing the availability of P_{org}. Some cover crop species seem to be capable of utilizing a *biochemical modification pathway*, where changes in the sorption capacity of the soil result in a greater quantity of plant-available phosphate. However, the latter pathway was apparently not important in the crop rotations used in our field experiments. The data also allowed us to characterize ways in which plant-soil-microbe interactions under cover crops affected the relationship of soil microbial functions to the enzymatic availability of P_{org} pools. Cover crops increased the abundance and activity of microbes, especially fungi, as well as microbial P. This enhancement in P-cycling potential shifted P_{org} toward pools of greater availability to added enzymes. However, the relation between enzymes and P_{org} pools is complex and is possibly affected by soil P composition and other site characteristics, indicating the need for further research in this area. Finally, we elucidated how the choice of cover crop species and agricultural management can shift the relative importance of the pathways for the P benefit of the main crop, while site-specific management allows farmers to adapt to local conditions and to optimize the functions of their agroecosystems.

In conclusion, our results indicate that the pathways of cover crop derived P benefit take place simultaneously. We confirmed the potential of cover crop biomass for the cycling of P, and we suggest that our observed increases in the availability of soil P_{org} are related to microbial abundance and activity. The interactions of cover cropping and tillage indicate also that P benefit can be optimized by management decisions. Finally, these new insights into soil phosphorus cycling in agroecosystems have the potential to support further development of more sustainable agricultural systems.

2 Zusammenfassung

Phosphor (P) ist einer der wichtigsten limitierenden Nährstoffe für das Pflanzenwachstum in der Landwirtschaft. Bodenmikroben spielen eine Schlüsselrolle in Nährstoffkreisläufen, beeinflussen das Wachstum von Pflanzenwurzeln, die Mobilisierung sowie die Mineralisierung von organischem P (P_{org}) und somit den Zugang zu P. Das Ziel dieser Dissertation war die Einschätzung des Potentials von Zwischenfrüchten zur Verbesserung der Interaktionen im System Pflanze-Boden-Mikroben und einer dadurch möglichen Steigerung der P-Verfügbarkeit für die Hauptfrüchte.

Diese Dissertation umfasst drei wissenschaftliche Veröffentlichungen. Die Literaturrecherche und die Meta-Analyse von 25 publizierten Feldversuchen ermöglichten es in Studie #1, ein Modell der Pfade der gesteigerten P-Verfügbarkeit nach Zwischenfrüchten zu präsentieren. Die selbst durchgeführen Feldexperimente der Studien #2 und #3 ergänzten das Verständnis der zugrundeliegenden Mechanismen mit besonderem Augenmerk auf die Rolle von Bodenmikroben. Die Feldexperimente wurden auf Lössböden mit Winterzwischenfrüchten vor Soja als Hauptfrucht in komplett randomisierten Blöcken mit vier Wiederholungen in Südwestdeutschland durchgeführt. In Studie #2 wurden die Wechselwirkungen zwischen Boden, Pflanze und Mikroben im Wurzelraum von drei verschiedenen Zwischenfrüchten (Senf, Phacelia und Buchweizen) in einem Boden mit geringer P-Verfügbarkeit untersucht. Studie #3 wertete die Interaktionen einer Zwischenfruchtmischung und Bodenbearbeitungsverfahren verschiedener Intensität mit ausreichender P-Verfügbarkeit anhand von Probennahmen in 0-5 und 5-20 cm Tiefe aus. In beiden Feldexperimenten wurde ein breites Set an bodenmikrobiologischen Methoden, inklusive Abundanz, Gemeinsschaftsstruktur von Bodenmikroben (Phospholipidfettsäuremuster, PLFAs), Aktivität von Enzymen des P-Kreislaufs und P in der mikrobiellen Biomasse mit der Verfügbarkeit von P_{org} für die enzymatische Mineralisierung in Zusammenhang gebracht. Zusätzlich wurden in Studie #2 die Abundanz von 16S-rRNA und *phoD*, einem Gen, das eine alkalische Phosphomonoesterase in Bakterien codiert, mit real-time qPCR quantifiziert. In Studie #3 wurde außerdem die Fähigkeit der mikrobiellen Gemeinschaft zur Nutzung von C-haltigen Substraten bestimmt.

Die verwendeten Methoden zeigten, dass Zwischenfrüchte den P-Gehalt in der mikrobiellen Biomasse, die Aktivität von Phosphatasen und mikrobielle Fettsäuremarker (PLFAs) erhöhen, was auf ein gesteigertes Umsatzpotential von organischen Phosphorverbindungen hindeutet. Die Abundanz von grampositiven und gramnegativen Bakterien, sowie in geringerem Umfang auch von arbuskulären Mykorrhizapilzen, wurde durch Zwischenfrüchte erhöht. Gleichwohl waren saprotrophe Bodenpilze die mikrobielle Gruppe, die am meisten von der Substratzufuhr der Wurzeln und Streu profitieren konnte. Stabiles P wurde in den aktiven Bodenzonen der Rhizosphäre und Detritusphäre in labilere P_{org}-Pools transformiert. Bewirtschaftungseffekte, wie die Wahl der Zwischenfrucht oder Bodenbearbeitung, waren erkennbar, aber wesentlich schwächer ausgeprägt als der Zwischenfruchteffekt insgesamt.

Unsere Ergebnisse bestätigen, dass Zwischenfruchtanbau zur Steigerung der P-Verfügbarkeit für die Hauptfrucht führen kann. Darüber hinaus konnten wir für den P-Vorteil drei grundsätzliche Wirkungspfade aufzeigen, die in aktiven Bodenräumen stattfinden. Über den *Wirkungspfad „Pflanzenbiomasse“* wird P aus dem Boden in die Biomasse der Zwischenfrucht aufgenommen und während der Zersetzung der Streu für die Hauptfrucht verfügbar. Über den *Wirkungspfad „mikrobielle Verstärkung“* steigert die Zwischenfrucht im Wurzelraum die Biomasse und Aktivität der mikrobiellen Gemeinschaft, wodurch diese die Verfügbarkeit von P_{org} erhöhen kann. Durch den Wirkungspfad „*biochemische Modifikation“* scheinen manche Zwischenfruchtarten in der Lage zu sein, über Wurzelexsudate die P-Sorption im Boden zu senken und dadurch den Anteil an pflanzenverfügbarem Phosphat zu erhöhen.

Weiterhin ermöglichen die erhobenen Daten die Diskussion, inwiefern mikrobielle Funktionen und die Mineralisierbarkeit von P_{org} zusammenhängen und wie die Interaktionen von Pflanzen beeinflusst werden. Zwischenfrüchte steigerten sowohl die Abundanz und Aktivität von Mikroben, als auch die Menge an P in der mikrobiellen Biomasse. Diese Potentialsteigerung des P-Kreislaufs steigerte die Verfügbarkeit des P_{org} für zugefügte Enzyme. Es muss bedacht werden, dass die Rückkopplungen zwischen Enzymaktivität und verschiedenen P_{org}-Pools komplex sind. Diese hängen von den lokalen Eigenschaften des

Bodens, wie etwa der Zusammensetzung des P-Vorrats, ab und sollten durch zukünftige Studien geklärt werden.

Drittens zeigen unsere Untersuchungen, wie die Wahl der Zwischenfrucht und die der Bewirtschaftung (z.B. Bodenbearbeitung oder Fruchtfolge) die relative Gewichtung der verschiedenen Pfade des P-Vorteils für die Hauptfrucht beeinflussen. Standortangepasste Zwischenfruchtsysteme erlauben es Landwirt:innen, die Funktionen ihres Agroökosystems hinsichtlich der lokalen Bedingungen zu optimieren.

Zusammenfassend bestätigen unsere Ergebnisse, dass der P-Bedarf der Hauptfrucht über die Biomasse der Zwischenfrucht gedeckt werden kann und zeigen auf, dass die charakterisierten drei Pfade des P-Vorteils durch Zwischenfruchtanbau parallel stattfinden. Schließlich können die hier gewonnenen Erkenntnisse über den Phosphorkreislauf, basierend auf der Kombination von bodenmikrobiologischen Methoden mit der Charakterisierung der Labilität von P_{org}, zur zukünftigen Entwicklung einer nachhaltigeren Landwirtschaft beitragen.

3 General introduction

Phosphorus (P) husbandry has long been a concern in agricultural sciences. Interestingly, while the use of green manures was described and encouraged even in antiquity by Greek and Roman authors (Winiwarter 2002), more modern agronomists have instead advocated mainly for the use of inorganic phosphate sources to maintain and increase yields (Heiden 1865). Currently, as humanity is approaching the global limits of nutrient cycling (Carpenter and Bennett 2011; Campbell et al. 2017), inefficient use of P fertilisers is depleting mineable reserves and creating environmental hazards (Alewell et al. 2020). Fortunately, over time, our understanding of P cycling has made significant progress, in particular by development of methods to assess soil organic P (P_{org}), largely through bettter characterization of the key role soil microbes occupy in P availability (McLaren et al. 2020). In this regard, soil-improving cropping systems that substitute biological functions for inorganic external inputs are increasingly important in the quest to secure future food supplies (Withers et al. 2018). The present work aims to shed light on the underlying processes supporting the great potential of microbial P cycling management through the incorporation of cover crops into agroecosystems.

The efficiency of P-fertilizers is low due to their differing interactions with the soil. As a result, only a very small percentage of soil P is present as plant-available phosphate in the soil solution due to physical (sorption onto particle surfaces, Fe- and Al-hydroxides) and chemical (production of secondary minerals such as Ca-apatite, or accumulation of recalcitrant P_{org} forms) interactions. Over-application of fertilizers in industrialised countries has led to the build-up of large soil P stocks in some areas (Menezes-Blackburn et al. 2018). Both of these, organic and sparingly available inorganic P pools, constitute important resources, however, and both could be managed with appropriate techniques.

Plants have developed a variety of mechanisms and strategies to access soil P. A substantial fraction of soil P, between 30 and 65 %, is present in organic forms (Harrison 1987) and is accessed by plants, frequently through their close interactions with microbes (Richardson and Simpson 2011). These strategies include increasing the colonized soil volume by optimizing root architecture and morphology (Honvault et al. 2021); formation of symbioses with arbuscular mycorrhizal fungi (AMF) in many plant species (Elbon and

Whalen 2015); mobilization of sorbed organic and inorganic P compounds, driven mainly by the release of H+/OH- and/or carboxylates by roots and microbes (Hinsinger 2001); and production of various phosphatases that hydrolyse enzyme-labile P_{org} producing plant-available phosphate. Microbes are an important source of these enzymes and play a crucial role in plant P nutrition (Richardson and Simpson 2011), while simultaneously providing for themselves a significant pool of relatively available P in the form of microbial biomass P (P_{mic}). Released rhizodeposits from plants are used by the microbes as C-sources, both shaping microbial communities and their capacity to increase access to P for the plants. Plant species differ greatly in their P acquisition strategies and interactions with the microbial community, however, underscoring the importance of aboveground biodiversity in agroecosystem nutrient management.

The technique of cover cropping is the practice of growing plants for multiple purposes in the intervals between cash crops' cultivation. Cover crop biomass remains on the field to reduce erosion, provide weed and pathogen control, enhance soil C inputs, and influence nutrient management (Marques et al. 2020). Cover crops improve access to P for main crops in multiple ways. For example, by means of a *biomass pathway,* available P can be taken up into the cover crop biomass and, as the biomass litter decomposes, become potentially available for the main crop (Damon et al. 2014). Additionally, enhancement of soil microbial abundance and activity by cover crops via a *microbial enhancement pathway* may also increase P availability in temperate agricultural soils by stimulating P_{org} cycling and facilitating access to P for the following main crop (Richardson et al. 2011). Lastly, some cover crops are able to biochemically modify their rhizosphere (Lambers et al. 2013), potentially increasing the solubility and availability of P for the main crop by means of a *biochemical modification pathway*. This general framework, together with a detailed description of soil-plant-microbe interactions involved in P cycling and plant P acquisition, is presented and discussed in Study #1, a meta-analysis of cover crop effects on P availability.

4 Objectives

In order to provide effective and efficient advice to farmers, we need a deeper understanding of the mechanisms and drivers controlling the underlying processes of cover crop-derived P benefits for the main crop.

There is current agronomic interest in improved nutrient management using cover crops, but more fundamentally, characterizing the effects of cover crop-induced changes on soil microbes and their regulatory functions provides an opportunity to study key aspects of terrestrial (organic) P-cycling. These include soil habitat conditions, such as the availability of root- or litter-derived C-sources and P sorption, as well as the abundances and activities of different microbial groups and enzymes involved in P cycling, all under the broader scientific umbrella of soil-plant-microbe interactions.

Therefore, the goal of this thesis is three-fold. First, it investigates whether and how cover crops increase access to P by main crops, specifically focusing on the role of soil microbes. Second, it elucidates the relationship of soil microbial functions to the enzymatic availability of P_{org} pools. Third, it clarifies how agronomic management (i.e., cover crop choice and tillage) affects plant-soil-microbe interactions. Deepening our knowledge of these mechanisms and approaches will enable us to improve the nutrient efficiency of agricultural systems in order to make responsible use of limited resources.

The studies presented here are among the first to thoroughly examine the mechanisms that influence cover crop-derived changes in P_{org} availability, providing a conceptual framework for greater understanding of the underlying processes. While measurement of enzymatic activities to quantify the mineralization potential of organic compounds is among the main tools for soil biologists (Burns 1982), quantification of the potential availability of different native P_{org} pools for mineralization with added enzymes is a relatively novel method (Bünemann 2008; Keller et al. 2012; Annaheim et al. 2013; Jarosch et al. 2015). Two field experiments, the simultaneous characterisation of the P_{org} pools with respect to their availability for added enzymes, coupled with assessment of enzymatic activities and other microbial properties, offer a novel and cutting edge approach providing deep insights into the *black box* P_{org}.

The first study in this thesis is a review and meta-analysis of cover crop-derived effects on P cycling. In this work, state-of-the-art understanding of P acquisition and soil-plant-microbe interactions is reviewed, with an emphasis on cover crop-related effects on P availability. Moreover, we present a conceptual framework with three distinct pathways (*plant biomass*, *microbial enhancement* and *biochemical modification*) for cover crops to affect P availability to the following main crops. The study further consists of a meta-analysis of 240 datasets derived from 25 different studies covering the results of field experiments examining cover crop biomass P content, main crop performance, and soil microbial properties. The hypotheses were: (1) P acquisition by cover crops stimulates growth and P uptake of different main crops; (2) cover crops enhance mycorrhizal colonization, short-term storage of P in soil microorganisms, and P mineralization, improving plant- and microbially-driven P uptake of the main crop; (3) site conditions (e.g., fractions of available P) modify P benefit to the main crop; and (4) cover crop management (i.e., species mixtures, tillage intensity, and fertilization) can be used to increase P benefits to the main crop.

The observed variability in results of the field experiments included in the meta-analysis required that we improve our mechanistic understanding of the availability of soil P_{org} pools. One of our principal aims, therefore, was to evaluate P dynamics under field conditions and to gain a more detailed understanding of the links between the functions of P cycling microbes and the lability of the corresponding P_{org} compounds.

A close look at soil geography indicates that the area around plant roots is among the most active soil compartments; intense plant-soil-microbial interactions here determine both the availability and use of different P pools (Honvault et al. 2021). In Study #2, the effects of different plant species on P-cycling enzymes' access to P_{org} pools around their roots was examined to investigate the mechanisms and implications of cover crop effects on soil microbial abundances and activities. Here we present the results of an on-farm field experiment assessing the microbial properties and P availability in the rhizosheaths of *Sinapis alba* (white mustard), *Fagopyrum esculentum* (buckwheat), and *Phacelia tanacetifolia* (purple tansy), grown as cover crops before *Glycine max* (soybean) on a soil low in available P. In order to evaluate the importance of the *biomass pathway*, the

quantities of P cycled through the cover crops' shoot and root biomass were assessed. We characterized abundances of microbial groups, the potential activity of P-cycling enzymes, and the lability of P_{org} pools to added enzymes. This enabled us to investigate the relationships among organisms producing enzymes, enzymatic activities, and the availability of P_{org} as a substrate. Additionally, the abundance of 16S-rRNA and the gene *phoD*, which codes for alkaline phosphomonoesterase in bacteria, were quantified using real-time qPCR. These data then tested the following hypotheses: 1) The selected cover crops increase labile P_{org} derived from microbial necromass or rhizodeposition in their rhizosheath. 2) Cover crop species differ in their plant-microbe interactions, leading to a distinct microbial community and activity in their rhizosheath. 3) The cover crops shape their rhizobiomes towards an increase in beneficial functions, e.g., by enhancing the specific enzymatic activity per unit of microbial abundance. And 4) Soybean as a subsequent main crop benefits from the increase in labile P_{org} and microbial activity by the cover crops.

Agricultural management affects plant-soil-microbe interactions and omission of this factor complicates the interpretation and comparability of studies. However, site-specific management allows farmers to adapt to local conditions and to optimize the functions of their agroecosystems. Currently, conservation agriculture is one of the main agricultural systems promoting the use of cover crops in combination with reduced tillage intensity. It is currently practiced by farmers in almost 80 countries extending over 200 million ha, corresponding to 15 % of annual cropland globally (FAO 2021). Tillage reduction is expected to benefit soil biota by decreasing soil disturbance and changes in plant litter distribution. This could be especially important with respect to the effects on organisms such as mycorrhizal fungi, which improve crop P uptake but may be negatively affected by tillage (Bowles et al. 2017). The interaction of tillage intensity and cover crop effects on soil microbes and soil P availability was therefore one of the main objectives of this thesis. In Study #3 we assessed the outcomes of a winter cover crop mixture and no-till on microbial properties. Microbial biomass, phospholipid fatty acids (PLFAs), P cycling enzymes, and carbon-substrate use capacity were linked with the lability of P_{org} pools. These results made it possible to examine the questions of whether: 1) conservation agricultural practices, such as cover crop mixtures and no-till, could shift soil P towards more available pools; 2) a

stimulated microbial community with enhanced functions is associated with changed P pools; and 3) cover crops and no-till may have synergistic effects on soil microbial biomass, microbial community structure, and P-cycling capacity.

5 Study #1: Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems

Hallama, M., Pekrun, C., Lambers, H. *et al.* Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant Soil* 434**,** 7–45 (2019). https://doi.org/10.1007/s11104-018-3810-7

Submitted: 27 April 2018; Accepted 06 September 2018

Published: 27 October 2018; Issue Date 15 January 2019

Moritz Hallama[1], Carola Pekrun[2], Hans Lambers[3], Ellen Kandeler[1]

[1] Soil Biology Section, Department of Soil Science and Land Evaluation, University of Hohenheim, Germany;

Corresponding author: hallama@uni-hohenheim.de;

[2] Agronomy Section, Institute of Applied Agriculture, Nuertingen-Geislingen University, Neckarsteige 6-10, 72622 Nürtingen, Germany

[3] School of Biological Sciences and Institute of Agriculture, University of Western Australia, 35 Stirling Highway, Crawley (Perth), WA6009, Australia

5.1 Abstract

Background Phosphorus (P) is a limiting nutrient in many agroecosystems and costly fertilizer inputs can cause negative environmental impacts. Cover crops constitute a promising management option for sustainable intensification of agriculture. However, their interactions with the soil microbial community, which is a key driver of P cycling, and their effects on the following crop, have not yet been systematically assessed.

Scope We conducted a meta-analysis of published field studies on cover crops and P cycling, focusing on plant-microbe interactions.

Conclusions We describe several distinct, simultaneous mechanisms by which P benefits the main crop. Decomposition dynamics, governed by P concentration, are critical for the transfer of P from cover crop residues to the main crop. Cover crops may enhance the soil microbial community by providing a legacy of increased mycorrhizal abundance, microbial biomass P, and phosphatase activity. Cover crops are generally most effective in systems low in available P, and may access 'unavailable' P pools. However, their effects on P availability are difficult to detect by standard soil P tests, except for increases after the use of *Lupinus* sp. Agricultural management (i.e. cover crop species selection, tillage, fertilization) can improve cover crop effects.

In summary, cover cropping has the potential to tighten nutrient cycling in agricultural systems under different conditions, increasing crop P nutrition and yield.

5.2 Introduction

Essential for agricultural production, but often limiting, mineable reserves of phosphorus (P) are non-renewable and concentrated in regions with territorial conflicts, adding a geopolitical dimension to P scarcity (Cordell and White 2014). Furthermore, P losses via erosion and leaching are responsible for eutrophication of water bodies and ecosystem degradation (Schoumans et al. 2014). Therefore, reliance on costly P-fertilizer inputs poses a threat to food security. Soil-improving cropping systems such as cover crops and conservation tillage are gaining attention for their potential to enhance overall sustainability of agriculture and P management (Tonitto et al. 2006; Simpson et al. 2011; Scopel et al. 2013; Damon et al. 2014). They play an increasingly important role with respect to the

concept of ecological intensification (Bommarco et al. 2013) and agroecology (Altieri 2002; Faucon et al. 2017).

Phosphorus-containing fertilizers are used extensively, but the processes underlying the biogeochemical P cycle are thus far not fully understood (Bünemann et al. 2011), especially under conditions of low P availability (Clarkson 1985; George et al. 2018). Phosphorus is present in soils in both mineral and organic forms with vastly different degrees of availability; only very small amounts of inorganic P (P_i) are present in the soil solution (Pierre and Parker 1927), and this is the form taken up by plants. In agricultural soils, the soil microbial community is increasingly acknowledged as the principal driver of soil P dynamics (Bünemann et al. 2011; Richardson and Simpson 2011), and efforts have been made to include soil microbes into P cycling models (Rengel 2008; Hinsinger et al. 2011; Damon et al. 2014). The relatively large pools of soil organic P (P_{org}), as a result of the combined action of plant- and microbial-exuded carboxylates for mobilization and enzymes for mineralization, constitute a valuable yet poorly understood resource (George et al. 2018; Menezes-Blackburn et al. 2018). The options for their management and their effects on crop P nutrition status and yield are therefore of paramount interest to both agronomists and farmers.

Cover cropping is the practice of growing plants, usually in the off-season, leaving their biomass on the field to provide various benefits for the agroecosystem, including erosion reduction, soil organic matter (SOM) build-up, weed and pathogen control, and nutrient management. Cover crops are also used to improve the P efficiency of added organic or mineral fertilizers by increasing soil biological activity or uptake and protection of soluble mineral P in strongly P-fixing soils (Kamh et al. 1998; Kuo et al. 2005). In principle, most plant species could be used for these purposes, and the list of plant species that can be used as cover crops is rapidly expanding. However, agronomic requirements of the cover crops (e.g., rapid growth, Plant species in general, and therefore also when used as cover crops, vary greatly in their biomass production, soil exploration, exudation of P-mobilizing and organic P-mineralizing compounds, as well as their interaction with the rhizosphere microbial community. The variety of strategies for P-acquisition employed by different plant types must be considered, as, e.g., non-mycorrhizal species, while highly efficient at P

mobilization, do not necessarily interact strongly with soil microbes (Lambers and Teste 2013; Lambers et al. 2015a). The inclusion of cover crops with special properties such as, e.g., the increase of the inoculum potential by arbuscular mycorrhizal fungi, can be beneficial in agricultural rotations that use domesticated cash crops developed in high-input breeding systems (Plenchette et al. 2005). Conservation agriculture, defined by the combination of cover crops, conservation tillage, and an adequate crop rotation (Hobbs et al. 2008), decreases labor intensity and frequency, enhances soil rest, and benefits soil biota. Here, crop residues are mixed less deeply into the soil than under conventional tillage, modifying soil biological parameters and mineralization dynamics. Other effects of management could result from the termination method used (e.g., spraying or roll-chopping) for winter-hard cover crops (Creamer and Dabney 2002).

The effects of cover crops and conservation tillage on crop yield and soil properties, especially nitrogen (N) dynamics, have been the subject of many studies, several reviews (Dabney et al. 2001; Dreymann et al. 2005; Tonitto et al. 2006; Dahlin and Stenberg 2009), and projects (Crossland et al. 2015). However, P dynamics have, until recently, rarely been addressed. Some reviews on the underlying mechanisms of plant and microbial P acquisition and resulting implications for agricultural management have provided a theoretical foundation for predicting the influence of cover crops on P dynamics (Horst et al. 2001; Guppy et al. 2005; Richardson and Simpson 2011; Richardson et al. 2011; Damon et al. 2014). Interestingly, those publications have largely reflected their regional conditions; climate, soil P content and sorption capacity. Studies of P-fertilization efficiency, often associated with pastures, come mainly from Australia (Rose et al. 2010a; Simpson et al. 2011; McLaughlin et al. 2011; Faucon et al. 2015). Brazilian studies have emphasized conservation agriculture and acid soils with poor P availability (LeMare et al. 1987; Calegari et al. 2013; Balota et al. 2014; Fageria et al. 2016; Varela et al. 2017), while Scandinavian researchers have concentrated on P leaching (Liu et al. 2015; Aronsson et al. 2016). In the USA, cover crops of different species have been investigated (Lal et al. 1978), including their effects on mycorrhizal fungi (Galvez et al. 1995; Zibilske and Makus 2009; Rick et al. 2011; Maltais-Landry 2015), whereas in China and India, studies have often focused on microbial inoculants (Devi et al. 2013; Cui et al. 2015). In some African countries, India, and Mexico,

with their prevalent traditional smallholder cropping systems, agroforestry and intercropping have been important topics for research (LeMare et al. 1987; Tarawali et al. 1999; Dinesh et al. 2004; Sileshi et al. 2008; Castillo-Caamal and Caamal-Maldonado 2011; Devi et al. 2013; Tanwar et al. 2014; Parihar et al. 2016). Previous meta-analyses have been concerned mainly with the effects of cover crops on *Zea mays* (maize) yield in North America (Miguez and Bollero 2005), effects on soil properties and yield in the South American Pampas region (Alvarez et al. 2017), response to woody and herbaceous legumes in sub-Saharan Africa (Sileshi et al. 2008), soil organic carbon (Poeplau and Don 2015). Nitrogen dynamics (Tonitto et al. 2006), P nutrition and dynamics has not been a focus of the meta-analyses in these agro-ecosystems. Consequently, understanding whether and how cover crops can benefit the P nutrition of following main crops is sorely needed.

The aim of our review is to bridge our present knowledge of soil-plant-microbe interactions with the potential of cover crops to stimulate P dynamics in agricultural ecosystems. We begin with a description of P pools and P-acquisition mechanisms of cover crops, and present a conceptual framework of how P dynamics of cover crops and main crops may be linked. Both plants and soil microorganisms are involved in P dynamics of agro-ecosystems; we therefore present the most important mechanisms and pathways for both with respect to cover crops. We reviewed the conceptual framework through an extended meta-analysis that included 240 datasets derived from 25 studies. The focus of the meta-analysis was to elucidate whether

(1) P acquisition by cover crops stimulates growth and P uptake of different main crops;

(2) cover crops enhance mycorrhizal colonization, short-term storage of P in soil microorganisms, and P mineralization, improving plant- and microbial-driven P uptake of the main crop;

(3) site conditions (e.g., fractions of available P) modify P benefit to the main crop; and

(4) cover crop management (i.e. species mixtures, tillage intensity, and fertilization) can be used to increase P benefits of the main crop.

1 Availability of phosphorus

Plant P nutrition is constrained by limited availability, due to physicochemical processes in the soil, of orthophosphate, the form that is taken up by roots. Phosphorus compounds

interact strongly with the soil through sorption to particle surfaces (including SOM), slow diffusion into aggregates, and formation of precipitates with cations of calcium (Ca), as well as sorption onto oxides and hydroxides of iron (Fe) and aluminium (Al) (Kelly and Midgley 1943), under alkaline and acid conditions, respectively. These interactions result in low P availability and P-fertilizer efficiency (McLaughlin et al. 2011). Soils with low P-sorption capacity also exhibit the associated hazard of P losses via leaching, whereas P-sorbing soils have problems with P-fertilizer efficiency due to immobilization of added P. As P-fertilizer efficiency is only 10-20 % in the short term (Chien et al. 2011), agricultural soils in industrialized countries have commonly received excessive loads of P over decades, often without reaching the soil saturation limit. This accumulated P constitutes a valuable resource that could be accessed by employing appropriate cropping systems. For a recent review about pools of recalcitrant P in agricultural soils and opportunities for mobilization, see Menezes-Blackburn et al. (2018). On the other hand, highly-weathered soils in the tropics are P impoverished, and rich in Fe/Al oxides and hydroxides (Simpson et al. 2011). In these systems, efficient recycling and use of the available inputs is essential. Phosphorus inputs into soil are from weathering of P-containing minerals or mobilization/mineralization of other P pools of low availability, as well as atmospheric deposition by dust, and by fertilizer application. Phosphorus is removed from the system through biomass of harvested crops, by erosion, and by leaching, and is accompanied by accumulation of P forms of low availability (Condron et al. 2005). Erosion, globally the biggest threat to sustainable soil stewardship (Bernoux et al. 2006; Durán Zuazo and Rodríguez Pleguezuelo 2008), is one of the major losses of agricultural P, as it is often associated with particle fractions prone to transport.

Soil P consists of both large, but stable and small, but highly-dynamic pools (Sharpley 1995). Although in heavily fertilized agricultural soils labile P_i may temporarily dominate the plant-available pools (Negassa and Leinweber 2009), usually 30-65 % of total P (P_t) is present in organic forms (Harrison 1987; Condron et al. 1990), and more in soils with high SOM content (Borie and Zunino 1983). During pedogenesis, the primary P-bearing minerals are slowly depleted, with highly weathered soils containing almost exclusively occluded P and P_{org}, which is tightly recycled in the biomass (Smeck 1985). Organic P can contribute to

crop nutrition, as plants and microbes can access the more labile P_{org} by a combination of mobilization with carboxylates and subsequent enzymatic mineralization (Condron et al. 2005; Richardson and Simpson 2011). In addition to phospholipids and nucleic acid-P (both forms account for less than 10 % of total soil P_{org}, but comprise most of the microbial P (P_{mic})), inositol phosphates (phytate) accumulate in soil due to their stable nature, and represent the major fraction of P_{org} (Jones and Oburger 2011). The inositol molecules consist of 1-6 phosphates attached to a C_6-ring with ester-bonds, requiring specialized enzymes for breakdown; they also interact strongly with the soil due to their high charge density (Turner et al. 2002; Turner 2007). Due to low substrate availability, low phytase production by roots, and low enzyme-substrate efficiency in soils, only some plant species can access phytate to a limited extent (Menezes-Blackburn et al. 2013). However, several plant species are able to grow with sodium (Na)-hexaphytate as their sole P source in the laboratory (Steffens et al. 2010). In natural soils with endogenous phytates, mobilization via carboxylates and subsequent interactions with microbes appear necessary for plants to use this resource. For a full review of inositol phosphates, see Turner et al. (2007).

The direction of the effect of long-term SOM accumulation by cover crops on P availability is not clear. Improved soil physical-chemical parameters (e.g., water-holding capacity, aggregate stability) (Dorado et al. 2003) may increase P availability to crops directly or indirectly (Eichler-Löbermann et al. 2008). Some fractions of SOM may compete with P for binding sites on particle surfaces, decreasing P-sorption capacity (Janegitz et al. 2013). However, large amounts of P_{org} are in the form of uncharacterized high-molecular-weight organic material (McLaren et al. 2015), linking the accumulation of poorly-available P_{org} closely to SOM dynamics. This could constitute a problem for the exploitation of these pools (Romanyà et al. 2017), as SOM is needed for soil structure, fertility, and climate change mitigation, and one target of cover cropping is to increase SOM content in soils. However, the fact that C:N:Sulfur (S) ratios are relatively constant across soils, whereas C:P and $C:P_{org}$ are more variable, may allow increasing available P (P_a) through P_{org} mineralization without affecting SOM accumulation. A P-priming effect has been described (Randhawa et al. 2005), but remains unresolved due to methodological constraints (Damon et al. 2014).

The most common way to characterize soil P is by using variants of the Hedley fractionation, which determines the amount of P_i and P_{org} in various soil extracts (i.e. $NaHCO_3$, NaOH, HCl), which are used to represent pools of differing degrees of availability (Hedley et al. 1982; Cross and Schlesinger 1995). However, the net contribution of these operational fractions to uptake by microbes and plants, and therefore the validity of the method for the prediction of plant P uptake, is not straightforward (Negassa and Leinweber 2009; Rose et al. 2010b). Olsen-P (Olsen et al. 1954), using $NaHCO_3$ as extractant, is one of the most widely used methods and often correlates well with yield and P uptake, but was originally developed for calcareous soils. There are many other extractants for soil P testing methods, including Mehlich-III (Mehlich 1984), Bray 1 (Bray and Kurtz 1945), water (Paauw 1971), calcium-ammonium-lactate (CAL) (Schüller 1969) and Colwell (Colwell 1963). Their application differs, even among regions in the same country, due to prevailing soil characteristics, but also due to historical reasons. More recently developed test methods that show promising results such as resin-P and "diffusive gradients in thin-films" (DGT) (Mason et al. 2013) are not yet widely used. One issue is that the color methods used in routine soil testing, mostly molybdate-blue (Murphy and Riley 1962), do not account for P_{org} (Steffens et al. 2010). This has been justified based on the assumption that P_{org} seems to play a minor role in plant nutrition under high availability of P_i (Guo et al. 2000), but has consequences for systems dependent on P_{org} (Dao et al. 2015). The combination of imperfect P-test methods and substantial knowledge gaps in understanding the complex P dynamics in soils constricts a scientific elaboration of general agricultural recommendations (Turner et al. 2005). Another problem is the definition of "plant-available P", as there are major differences among plant species (Lambers et al. 2006) and even crop varieties (Pang et al. 2018a) regarding their ability to access different soil P pools.

2 Phosphorus acquisition by cover crops

Plant species differ greatly in their P-acquisition strategies. The ability of cover crops to access poorly-available soil P is often superior to that of cash crops (Fig. 5.1). Their strategies can be summarized as: 1) exploration of a greater soil volume by an adaptive root architecture and root morphology; 2) mobilization of sparingly-soluble inorganic and organic

P forms; and 3) mineralization of P_{org}. These mechanisms may all be enhanced through interaction with soil microbes.

In addition to the transfer of P_a via cover crop residues to the main crop and to chemical rhizosphere modifications, some studies attribute benefits of cover crops to the subsequent crop to the soil microbial community, which influences P dynamics, both during the cover crop and the main crop phases (Nuruzzaman et al. 2005a; Pypers et al. 2007; Rose et al. 2010a; Mat Hassan et al. 2013).

Plants interact with the soil microbial community by releasing organic compounds into the rhizosphere that serve as substrates and signaling molecules to the microbes, increasing their abundance and activity several-fold (Bünemann et al. 2004; Balota et al. 2014). Our current knowledge indicates that both plants and soil determine microbial community composition (Marschner et al. 2001; Nannipieri et al. 2008). Together with plant roots, microbes are the principal drivers of P_i and P_{org} solubilization and of the mineralization of more or less recalcitrant P_{org} in soil. They possess a diverse array of mechanisms to increase P acquisition by plants, including modifications and extension of root systems, allowing roots to access P-rich substrates that are otherwise unavailable to plants due to their location in the soil (i.e. in narrow pore spaces).

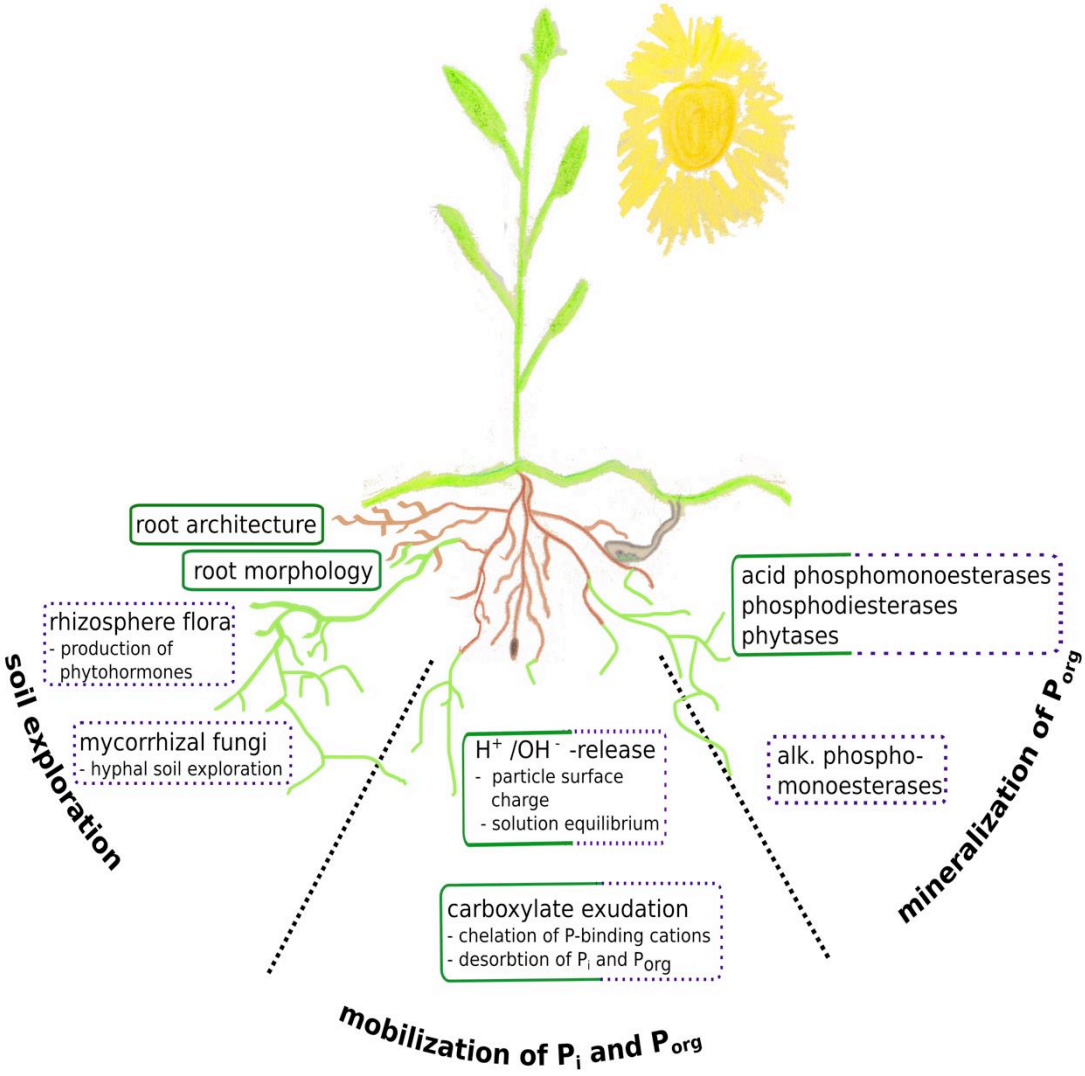


Figure 5.1 Strategies and mechanisms for phosphorus (P) acquisition by plants: 1) soil exploration via roots and mycorrhizal hyphae; 2) mobilization of sparingly-soluble inorganic P (P_i) and organic P (P_{org}) by exudation of H^+/OH^- and carboxylates; 3) mineralization of P_{org} by phosphatases. Plant-driven processes have solid outlines, microbial activity is shown by dotted outlines

3 Root architecture

The differences in P uptake by cover crops are determined partly by their root architecture, with topsoil exploration and root hair density the most important traits for improved P uptake (Richardson et al. 2011). The kinetic properties of the P_i-uptake system, unlike those of more mobile nutrients such as nitrate, are not a major rate-limiting step in plant P acquisition (Clarkson 1985; Barber 1995). Mycorrhizal fungi play a fundamental role as extensions of the roots, whereas other microorganisms promote root growth and modify

root architecture (branching, root hairs) via signaling molecules in the rhizosphere (Hayat et al. 2010). Cover crops with more extensive root systems scavenge P from a larger and deeper soil volume, and make it potentially available for main crops with shallow roots (Dube et al. 2014). Some plants (e.g., *Lupinus* species), possess specialized root structures, termed *cluster roots*, that exploit soils with low P availability and potentially enhance P availability for the main crop (Nuruzzaman et al. 2005b; Lambers et al. 2006). They may additionally facilitate P acquisition for neighboring plants (Gardner and Boundy 1983; Horst and Waschkies 1987; Cu et al. 2005).

To date, the most extensive description of cover crop traits related to P uptake is that by Wendling et al. (2016), who classified cover crops into five groups based on shoot biomass and nutrient concentration, comprising species from different families. The main findings of this study were shoot and root traits, rather than taxonomy; species with high nutrient concentrations and high root length density were recommended under high-fertility conditions and from a short-term perspective. However, although biochemical and microbial root P-acquisition strategies were not assessed in this study, they may well have been relevant, especially in systems with low P availability.

4 Phosphorus mobilization

Soil P-mining strategies enhance desorption and solubilization of sparingly-available P_i and P_{org} pools, which often limit P availability. Plants and microbes are capable of exuding low-molecular-weight organic anions (carboxylates) to dissolve precipitates and chelate metal cations, both of which make phosphate unavailable; because carboxylates facilitate the release of sorbed P via ligand-exchange reactions (Hinsinger 2001), and block binding sites on soil particles, they increase the concentration of P in solution (Ohno and Crannell 1996). The pH of the soil solution is modified by exudation of H^+ or OH^-/HCO_3^-. This, in turn, determines the variable surface charge of minerals and SOM, and may also increase P in solution. Proton release enhances P availability only in calcareous soils, due to the dissolution of Ca-phosphate. However, as the (bio)chemistry of P in soils is very complex, with many processes occurring simultaneously, sometimes in opposite directions, it is difficult to predict the effect of pH changes on P dynamics. A review of P_i bioavailability in

the rhizosphere was written by Hinsinger (2001). Despite their name, 'organic acids' do not substantially decrease rhizosphere pH, as they are mostly released as organic anions, generally with cations other than protons as balancing ions (Zhu et al. 2005; Roelofs et al. 2008). Exudation of P-solubilizing organic anions differs strongly among plant genotypes and soils (Kamh et al. 1998; Nuruzzaman et al. 2006) and is related to P deficiency and Al toxicity (Richardson et al. 2011). Citrate is a commonly released organic anion and one of the most effective for P mobilization (Jones 1998). It is produced in large quantities by *Lupinus albus* (Gardner et al. 1983; Dinkelaker et al. 1989; Cu et al. 2005) and other legumes (Kamh et al. 2002), including *Cicer arietinum* (chickpea) (Veneklaas et al. 2003), *Vicia faba* (faba bean) (Li et al. 2007), and *Trifolium pratense* (red clover) (Gerke and Meyer 1995), but also by *Brassica napus* (canola) (Hoffland et al. 1992). The strategy of dicots (e.g., Fabaceae, Brassicaceae) is to utilize biochemical rhizosphere modification for P mobilization, whereas Poaceae predominantly take up P using their extensive root systems (Maltais-Landry 2015; Schnug and De Kok 2016). The exudation of different organic anions and acidification may be complementary (Gerke and Meyer 1995), but the mechanisms are complex and the result depends strongly on soil chemistry and P level (Oburger et al. 2011). Complicating the system further, microorganisms function as both potential sinks and alternative sources of carboxylates (Deubel et al. 2000), and soil fauna remobilize P from the microbial biomass (Hinsinger et al. 2015). The identification of pH as the principal driver of microbial diversity in soils (Philippot et al. 2009) paired with substantial changes in pH in the rhizosphere led Hinsinger et al. (2009) to the hypothesis that root-induced pH changes shape the structure of the rhizosphere microbial community equally or more importantly than root C deposition.

Microbes may use plant exudates to produce P-solubilizing compounds in the rhizosphere, complementing P mobilization by roots (Schilling et al. 1998). In fact, some root exudates do not function directly in plant nutrient acquisition, but are composed of mobile sugars, which can be used by the rhizosphere microbial community. The critical role of desorption for the mineralization of P_{org} is receiving increasing attention (Giaveno et al. 2010). *Pseudomonas* species are among the most frequently studied P-solubilizing bacteria, but also species of *Burkholderia*, *Enterobacter*, *Pantoea*, *Bacillus* solubilize P (Jorquera et al.

2008). Together with arbuscular mycorrhizal fungi (AMF), P-solubilizing microorganisms are a target for the development of microbial inoculants, although often with limited success in the field (Parray et al. 2016).

The potential of some plants to mine sparingly-available P pools led to optimism about their potential to increase P availability when used as cover crops (Teboh and Franzen 2011; Boglaienko et al. 2014). However, under conditions of P-deficiency, plants do not always respond by releasing organic anions and their effect on P uptake is not consistent (Wang et al. 2016).

The minor direct benefits of cover crop rhizosphere modification (i.e. carboxylate exudation) for subsequent crops (Possinger et al. 2013) may be explained by the short duration of carboxylate exudation (a few weeks) associated with legume roots, limiting their effects on the main crop (Nuruzzaman et al. 2005a). Notwithstanding, the binding of carboxylates to Fe/Al hydroxides could delay microbial mineralization (Jones and Edwards 1998) and reduce soil P-sorption capacity. The ability of microorganisms to access sparingly-available P with benefits to plants may depend on microbial turnover (Richardson and Simpson 2011), as the microbial biomass conserves solubilized P.

The inclusion of carboxylates and other rhizosphere processes could improve plant nutrition models, which have to date failed to predict the actual uptake of P and other low-mobility nutrients, especially under low-input conditions (Hinsinger et al. 2011). Leaf manganese (Mn) concentration, for example, can be used as a proxy for carboxylate concentration in the rhozosphere, providing a tool that may be more reliable than measurement of rhizosphere carboxylates, given their transient nature in the field (Lambers et al. 2015b; Pang et al. 2018a).

5 Mineralization

In addition to mobilization mechanisms described above, the considerable amounts of P_{org} in soil require, once in solution, enzymatic hydrolysis to become plant-available P_i. In pot experiments, the activity of phosphatase enzymes was three-fold and nine-fold greater in the rhizosphere of *Triticum aestivum* (wheat) and *Lupinus albus* (white lupin), respectively, than in bulk soil, and this increased activity was, in both cases, associated with

the depletion of soil P_{org} (Nuruzzaman et al. 2006). However, phosphatases also have a role in recycling P inside cells and recapturing P_{org} lost from roots or microbial cells (Tarafdar and Jungk 1987; Barrett-Lennard et al. 1993). Experiments with transgenic *Trifolium repens* have shown that, under laboratory conditions, transgenic expression of phytase and purple acid phosphatase genes from *Medicago truncatula* increased the plants' ability to utilize organic P in response to P deficiency (Ma et al. 2009). The use of these techniques is convenient for experimentation, but in the field, the efficacy of single exudation traits appears to be limited in P-deficient soil conditions where the soil does not exactly match the functional requirements of the enzymes of interest (Giles et al. 2017).

Extracellular enzymes interact strongly with soil particles, leading to adsorption and inactivation, but also to protection against degradation (Rao et al. 2000). Adsorption depends on the mineral composition of the soil (Ditterich et al. 2016) and characteristics of the SOM. There are indications that carboxylates may serve a dual role of desorbing P and providing a favorable pH for the phosphatase enzymes, increasing enzymatic activity (Furutani et al. 2017)

Due to sorption processes, the effect of plant-derived phosphatases will be restricted to a few millimeters of distance from the roots. However, mobile rhizodeposited sugars penetrate further into the bulk soil, and can be used by microbes to produce phosphatases, extending the range of P_{org} mineralization around the roots. Due to complex interactions with soil, microbial degradation, and interception of the products, increased phosphatase activities do not necessarily translate into a more rapid P-uptake rate for plants.

Under natural conditions, the microbial contribution to the mineralization of P_{org} in the rhizosphere is undisputed; however, it is often difficult to separate the origin of enzymatic activity, as some enzymes, such as acid phosphatases and some phytases, are produced both by plants and microbes (Nannipieri et al. 2011). Diesterases can be produced by plants also, but diesterase activity is mainly related to microbial biomass (Turner and Haygarth 2005; Lang et al. 2017). Alkaline phosphatases, however, some phytases (Azeem et al. 2015) and phosphonate hydrolases (Hunter et al. 2014) are produced only by microbes. Zymography is a promising *in situ* method for analysis of the two-dimensional distribution of enzymatic activity in soil. In an experiment with *Lupinus albus* it was combined with ^{14}C

imaging, revealing that alkaline phosphatase-producing microorganisms were not dependent on recent rhizodeposition, whereas acid phosphatase activity was concentrated in the direct vicinity of the roots (Spohn and Kuzyakov 2013a). The relative contributions of microbial groups to the activities of the different phosphatases requires further investigation (Turner and Haygarth 2005). In contrast with plants, which take up P exclusively as P_i, microorganisms may be able to take up low-molecular-weight P_{org}, and protozoa can make use of high-molecular-weight P_{org} (Jones and Oburger 2011).

The capacities of a plant species to solubilize and mineralize P_{org} forms may be related to its rhizosphere-associated microbes. For example, the pasture plants *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover) have predominantly phytate-mineralizing bacteria in their rhizospheres, whereas in the cereal crops *Avena sativa* (oat) and *Triticum aestivum* (wheat), P-solubilizing bacteria dominate in the rhizosphere. Conversely, *Lupinus luteus* (yellow lupin) shows the lowest proportion of both bacterial types in a Chilean volcanic soil (Jorquera et al. 2008). Larger quantities of phytate-mineralizing and P-solubilizing fungi can be isolated from the rhizosphere of leguminous crops as compared with those of cereals (Gaind and Nain 2015). The ecological interactions between *r*-strategists in the rhizosphere and *K*-strategists in the bulk soil may also influence P mineralization (Hunter et al. 2014).

Strategies used by plants vary. There is a rather microbe-independent strategy, as in *Lupinus albus,* which releases inhibitors that prevent microbial degradation of root-derived carboxylates and phosphatases, intensively changing the chemistry of a small volume of soil around the cluster roots (Weisskopf et al. 2006). However, there are also non-mycorrhizal Brassicaceae with high levels of rhizodeposition, and mycorrhizal plants that scavenge P from a greater soil volume at a lower intensity. Therefore, the P-acquisition strategy of the cover crop influences the mechanisms of P-benefit to the main crop. A selection of common cover crop species and their properties is described in Table 5.1.

Table 5.1: Plant species widely used for cover cropping and their properties as described in the literature

Cover crop	Advantages	Disadvantages	P(1) -acquisition strategy	Cited in
Vicia faba (faba bean) Fabaceae	• N_2(2) fixation • P mobilization		• Rhizosphere modification: pH, carboxylates, phosphatases)	Nuruzzaman et al. (2005b) Rose et al. (2010) Malthais-Landry (2015)
Vicia villosa (hairy vetch) Fabaceae	• High yielding • Cold tolerant	• Mixed effect on AMF(3)		Anugroho et al. (2009) Tarui et al. (2013) Mbuthia et al. (2015)
Lupinus sp. (lupin) Fabaceae	• Excellent P mobilization • N fixation	• Non-mycorrhizal • Difficult establishment	Cluster roots: intensive exudation of carboxylates, protons and enzymes	Veneklaas et al. (2003) Lambers et al. (2013) Janegitz et al. (2013)
Lolium sp. (ryegrass) Poaceae	• Good nutrient scavenger • Erosion and weed control • Cold tolerant	• High C:P ratio • P immobilization	• Extensive root system	Aronsson et al. (2016)
Avena sativa (oat) Poaceae	• Fine rooting system, competitive • Winter kills			Muzangwa et al. (2012) Mukumbareza et al. (2015)
Secale cereale (rye) Poaceae	• Fast growth • Good nutrient scavenger • Late sowing possible • Cold tolerant	• Nutrient immobilization • Termination difficult		White and Weil (2010) Maltais-Landry (2015)
Brachiaria sp. (ruzigrass) Poaceae	• High biomass		• Decreases P-sorption of acid soils • Converts recalcitrant P into available P	Janegitz et al. (2013) Almeida and Rosolem (2016)
Sinapis sp. (mustard) Brassicaceae	• High biomass • N and P scavenger • Taproots • Biofumigation	• Non-mycorrhizal • Poor improvement of soil structure	• Rhizosphere modification (phosphatases, carboxylates), but no strong acidification • High biomass	Haramoto and Gallandt (2004)
Fagopyrum esculentum (buckwheat) Polygonaceae	• Fast growing • P scavenger (carboxylates) • Winter kills	• Non-mycorrhizal • Weed hazard when allowed to set seed • Low root biomass	• Organic anion and proton release • Good solubilization of Ca-P(4)	Teboh and Franzen (2011) Boglaienko et al. (2014)

(1) Phosphorus; (2) Nitrogen; (3) Arbuscular Mycorrhizal Fungi; (4) Calcium-Phosphate

Conceptual framework: how do cover crops affect P dynamics?

Among the P dynamics affected by the soil-plant-microbe processes of cover cropping (Fig. 5.2), the most studied mechanism is the direct uptake of P_a by plants and the transfer of P within the cover crop biomass. The storage of substantial quantities of P, both by high biomass and high P concentrations, is methodologically relatively simple to assess. Phosphorus in the plant biomass is protected from sorption onto the soil (Groffman et al. 1987) or losses by erosion and leaching, but P mineralization needs to be in synchrony with the needs of the main crop. Some cover crops act through positive effects on the soil microbial community (i.e. earlier mycorrhizal colonization, production of enzymes and increased microbial P as a pool for plants), increasing the capacity of the crop-rhizobiome-system to access P (Njeru et al. 2014). There is also chemical modification of the rhizosphere, via changes in pH, carboxylate exudation, or phosphatase release, as described above. Biochemical P mobilization would be potentially greatest in soils with a high content of poorly-available P, under the condition that chemical modification of the rhizosphere persists well into the main cropping phase. All these processes occur simultaneously with differing degrees of relative importance depending on the combination of agroecosystem and management.

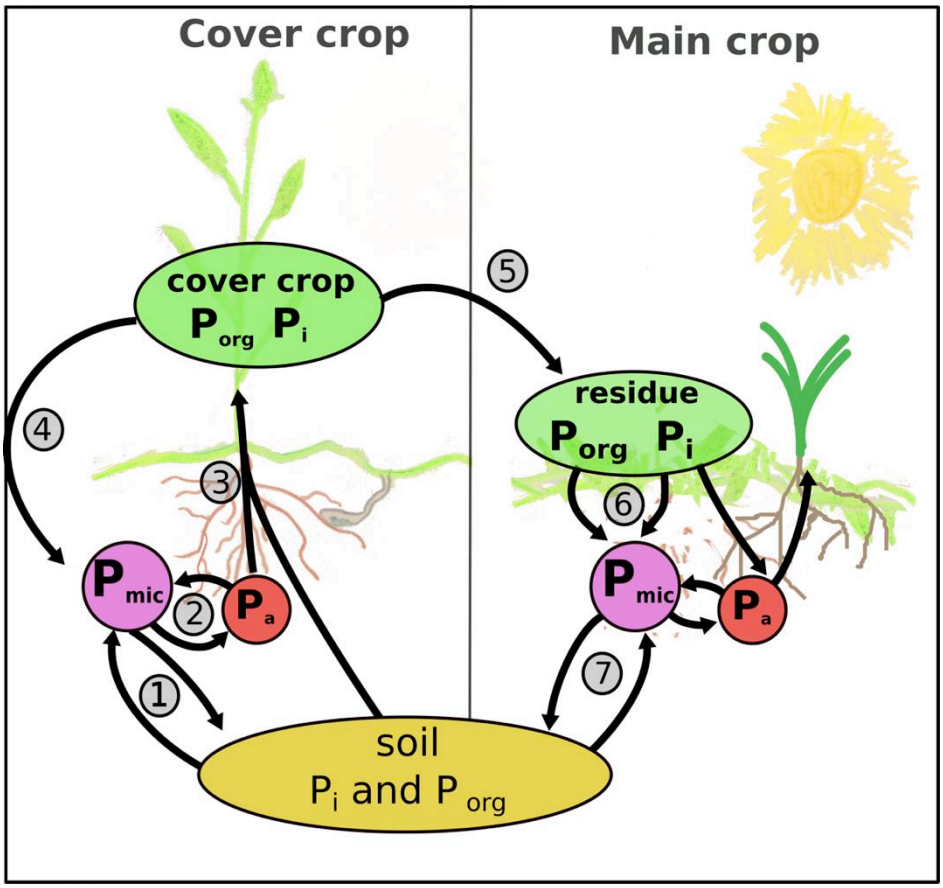


Figure 5.2 *Pathways of phosphorus (P) transfer and plant-microbial processes affecting P availability by cover cropping. 1) Soil P pools of varying degrees of availability are solubilized and/or mineralized and are immobilized in the microbial biomass. 2) The microbial biomass releases P into the soil solution which 3) ends up in the plant via root or mycorrhizal uptake. Cover crops may additionally possess the capacity to mine P from poorly-available P pools or to produce biochemical rhizosphere modifications to increase P availability. 4) The roots release rhizodeposits that shape the microbial community, eventually leading to increased P mining. 5) The P stored in the cover crop biomass is transferred to the main crop via cover crop residues, which are decomposed by the soil microbial community (6). The soil microbial community (i.e. mycorrhizal fungi) in the main crop phase, enhanced by the cover crops, may possess an increased capacity to mine P for the main crop (7).*

5.3 The Meta-Analysis

In order to analyze the general effects of cover cropping on main crop performance in terms of P nutrition, we conducted a meta-analysis. We also assessed more specific effects, such as the multiple ways in which cover crops, interacting with microbes, influence P dynamics and P uptake of the main crop, as well as different cover crop-main crop combinations.

An initial search in 2017 for online available publications using Scopus with the key-words (”phosphorus“ AND "cover crop" OR "green manure" OR "catch crop") yielded 638 matches that were screened by title and abstract. The literature cited in the studies meeting our criteria was also screened, and we expanded the search further using Google Scholar. We selected those studies that reported the effects on main crop yield and P uptake/P concentration, soil P and/or soil biological parameters related to P cycling (phosphatase activity, microbial biomass P, or abundance of AMF) and included a control treatment without cover crops. Phosphorus-mobilizing carboxylates are rarely measured in field studies and could not be included in the meta-analysis. We used only studies with cover crops and main crops grown in rotation, excluding intercropping or living mulch. Greenhouse experiments were excluded, as were agroforestry and grassland studies. Soil biological properties and available P were determined after termination of the cover crop or during growth of the main crop. Experimental factors such as main crop species and/or other factors (e.g., soils, tillage) and data from different years were treated as separate experiments within a study.

The soils included in this meta-analysis were classified according to their P availability, using the descriptions of the field experiments and the results of standard P tests. Datasets from a single field experiment that had been published in several articles (e.g., yield and soil microbiology in different papers) were merged into a single dataset when possible. Details of the studies used (Weerakoon et al. 1992; Medhi and Datta 1996; Boswell et al. 1998; Vanlauwe et al. 2000; Kabir and Koide 2002; Somado et al. 2003; Jensen et al. 2005; Rutunga et al. 2008; Wang et al. 2008; Eichler-Löbermann et al. 2008; Oikeh et al. 2008; Takeda et al. 2009b; White and Weil 2010; Buyer et al. 2010; Rick et al. 2011; Tiecher et al. 2012b, a; Karasawa and Takebe 2012; Njeru et al. 2014; Balota et al. 2014; Maltais-Landry et al. 2015; Karasawa and Takahashi 2015; Mbuthia et al. 2015; Ro et al. 2016; Pavinato et al. 2017) and the extracted data can be found in supplementary material S5.1 and S5.2). The data were extracted from the publications using the shareware-tool *DataThief III* (Tummers 2006) and the open source software *Tabula* (Aristarán et al. 2017).

We used *main crop yield* and main crop *P uptake* as response variables to evaluate the effect of cover crops, because yield is ultimately of interest to farmers. We decided against

P concentration, because there is a trade-off between yield and nutrient concentration; that is, high-yielding cropping methods may decrease the concentration of some minerals in the crop (Garibay et al. 1997), whereas a crop with poor field emergence may have a high P concentration. *Cover crop biomass* and *cover crop P uptake* were also evaluated to characterize the different cover crop families. To assess the interactions between cover crops, soil microbial community, and the main crops, *AMF abundance/colonization,* P_{mic}, and *phosphatase activity* (alkaline and acid phosphomonoesterase, phosphodiesterase) were first treated as response variables to determine whether or not they were affected by cover cropping, then included in a separate analysis as moderating variables to determine their influence on main crop performance.

The categorical variables *soil P availability* (high vs low) and *climate* (tropical vs temperate), and the agronomic factors *tillage* (inversion tillage vs non-inversion tillage/no-tillage), *fertilization* (P-fertilized vs unfertilized), and *cropping system* (conventional vs organic) were also used as moderating variables for the response variables.

The models had the following basic structure with their respective response and moderating variables:

$$\log_e(\hat{y}) = m\ (fixed\ ) + study(fixed) + study{:}\ experiment(nested, random)$$

y = response variable

m = moderating variables

The response variables were all $\log_e$-transformed to account for different units and scale effects, but back-transformed and reported as percentage change relative to the respective control treatments for graphical visualization. To calculate the relative percentage change by cover crops, the following formula was used:

$$Y(\%) = \frac{\hat{y}_{cover\ crops_j} - \hat{y}_{control_j}}{\hat{y}_{control_j}} * 100$$

$\hat{y}_{cover\ crop_j}$ = modeled median or 95 % CI, respectively, of the *j*th cover crop type

$\hat{y}_{control}$ = modeled median of the control treatments corresponding to *j*

Bearing in mind possible interactions, cover crops and main crops were aggregated into phylogenetic families of similar properties (Supplementary Material S5.2). Fabaceae and Poaceae cover crops were by far the most studied groups. *Lupinus* sp. were not included in the Fabaceae group due to the special P-mobilizing properties of this non-mycorrhizal genus (Lambers et al. 2013). *Phacelia* (Hydrophyllaceae, only tested in one study) was included in the Asteraceae family, due to similarities in their respective mycorrhizal competence and biomass production. Despite promising results, cover crop mixtures, usually consisting of a Poaceae and either a Brassicaceae or a Fabaceae, were seldom assessed.

Linear mixed models with *study* as fixed effect and the interaction of *study* and *experiment* as random effect were fitted using the package *lme4* v1.1-15 (Bates et al. 2015, p. 4) in *R* v3.4.3 (R-Core Team 2013) and *R-Studio* v1.1.423 (RStudio 2013). Graphs were produced with the packages *ggplot2* v2.2.1 (Wickham 2009) and *cowplot* v0.9.2 (Wilke 2017) with estimates from *emmeans* v1.1 (Lenth 2018) and percentages calculated with *plyr* v1.8.4 (Wickham 2011). As variance or related parameters were not reported in several studies, the observations were weighted by the number of replicates in each experiment with the *weights*-statement in the *lmer* function (all studies had a balanced design). Different models were compared using ML estimation, whereas the final models were fitted with REML. The structure of the fitted models and the F-tests obtained with the package *lmerTest* v2.0-36 (Kuznetsova et al. 2016) are provided in Supplementary Material S5.3, sample R code in Supplementary Material S5.4.

There was a large variance in and among the published studies due to differences in climate, site conditions, experimental set-ups and management, but also substantial intra-study heterogeneity. Because of missing factor combinations (e.g., not all cover crops were

grown with fertilization or tillage), we encountered some difficulties in accounting for interactions of factors. In some cases, we opted to use models with fewer interactions and a higher punctuation by Aikaike's Information Criterion (AIC) in order to be able to use a greater part of the dataset, under the condition that this did not substantially distort the model output. For the same reason, the moderating variables were tested in separate models. Main crop yield and P content do not represent exactly the same dataset, because not all studies reported both variables. In the studies with wetland rice, only Fabaceae were used as cover crops, so the yield and P uptake of this main crop were calculated separately.

5.4 Results of the Meta-Analysis

6 Cover crop biomass and P content

From the analysis of the aggregated data from the studies included in the meta-analysis it can be seen that the selection of cover crop was a relevant factor. Cover crop type determined the biomass produced and the tissue P concentration (Fig. 5.3, Supplementary Material S5.3.1: Models 1.1-3.3). Biomass and P concentration were not correlated, resulting in differing C:P ratios: Poaceae cover crops produced the most biomass, but had the lowest P concentration; Polygonaceae had the lowest biomass and Fabaceae and Brassicaceae had rather high P concentrations.

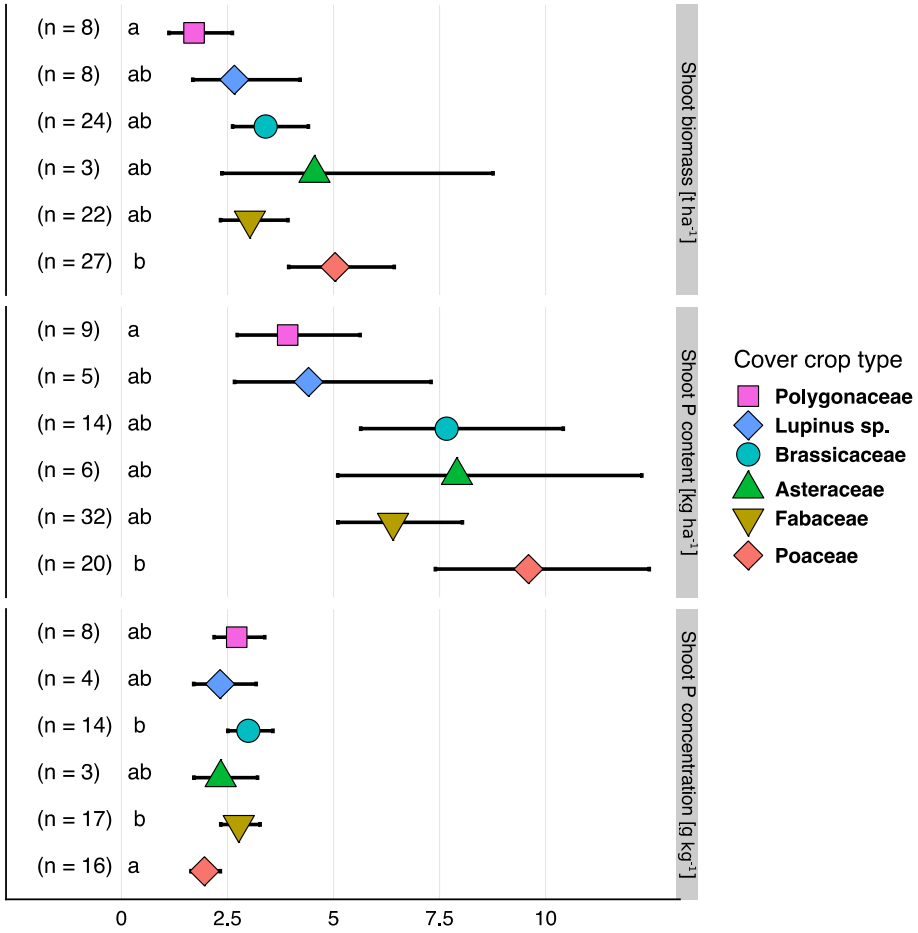


Figure 5.3 *Cover crop dry matter biomass [t ha^{-1}], shoot phosphorus (P) content [kg ha^{-1}] and concentration of P in biomass [g kg^{-1}]. The points represent the modeled median (+/- 95 % CI) of the different cover crop treatments. On the left are displayed the number of observations. The letters indicate significant differences among cover crop types with a Tukey post-hoc test (p<0.05). The corresponding models can be found in Supplementary Material S5.3.1*

7 Crop rotation

The integration of cover crops into crop rotations generally increased main crop yields (Fig. 5.4; Supplementary Material S5.3.2: Models 2.1 and 4.2). Main crop yield benefit was determined by main crop species, cover crop type, and their interaction. Maize was most responsive to cover cropping. Other main crops (i.e. *Glycine max* (soybean) and cereals) tended to respond positively, but the increases were not significant. Wetland rice yields were significantly enhanced by Fabaceae cover crops (Supplementary Material S5.3.2: Model 2.3). Brassicaceae, vegetables, and cotton (aggregated as *other* main crops) were

tested in few studies with little response to cover cropping. The interaction *cover crop type* x *main crop type* improved the model significantly, although the F-test was not significant. Main crop P uptake was closely related to yields (Fig. S7.5).

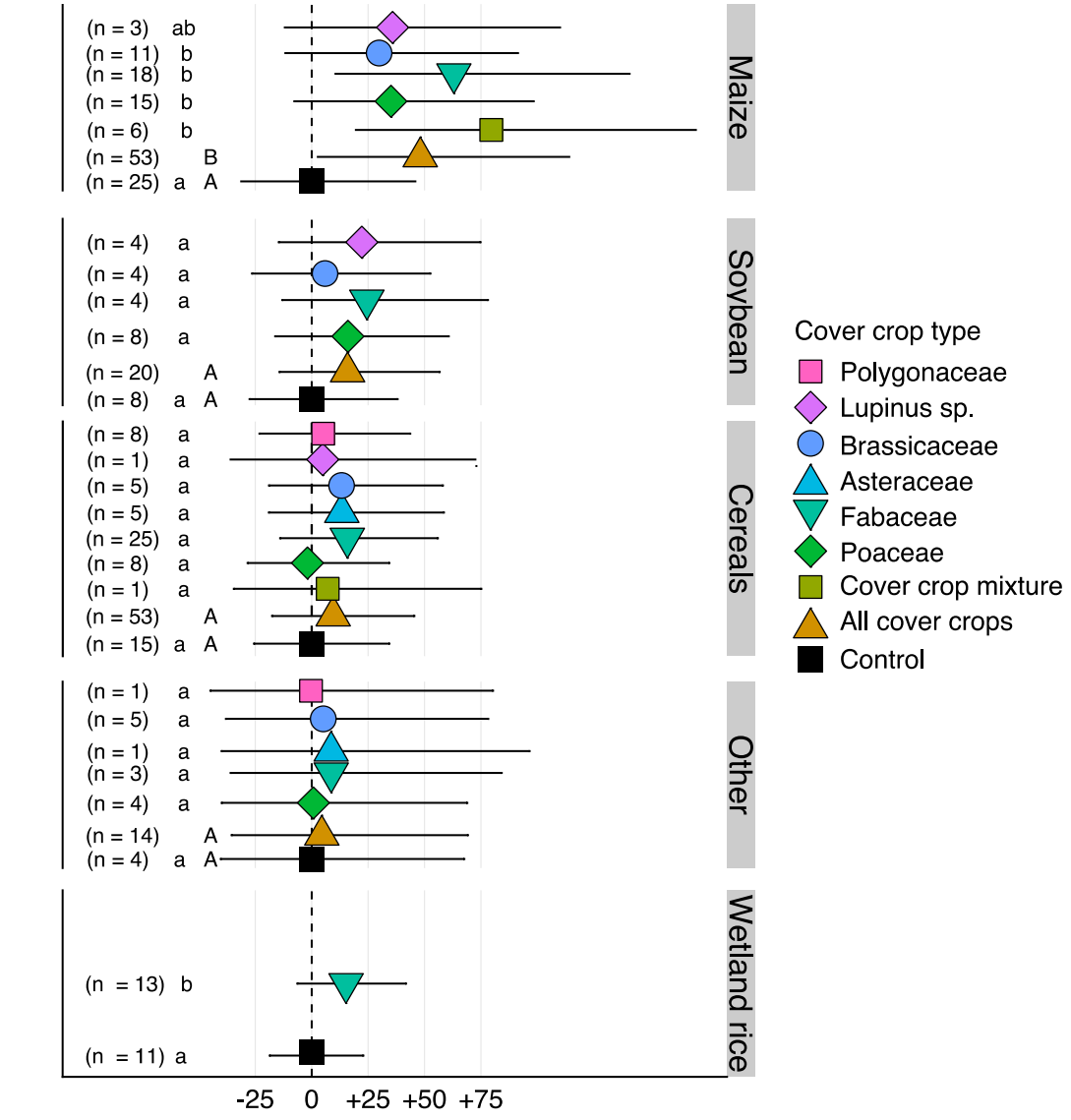
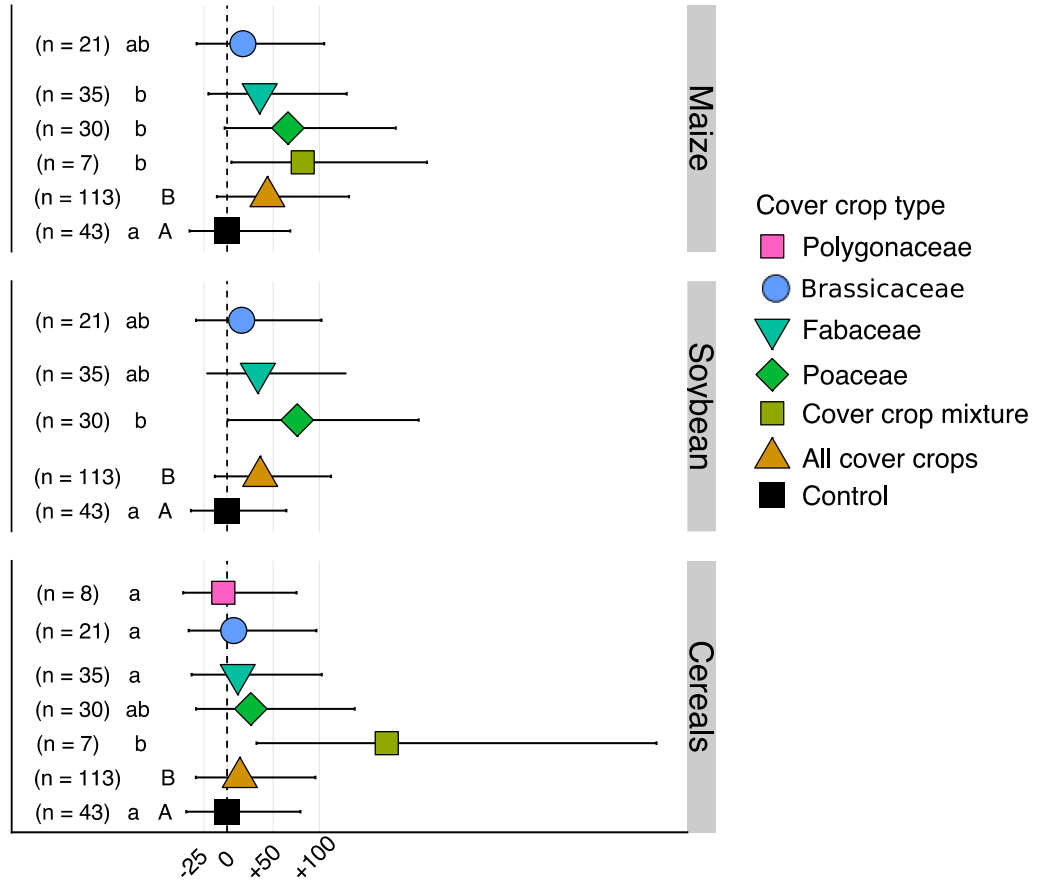


Figure 5.4 *Change in main crop yield and shoot biomass following cover crops from different families. The points represent the modeled median (+/- 95 % CI), relative to the respective controls. On the left are displayed the number of observations. The lower-case letters indicate, for a single main crop type with a Tukey post-hoc test ($p<0.05$), significant differences among cover crop types (including the control) and the upper-case letters between cover cropping in general and the controls. The corresponding models can be found in Supplementary Material S5.3.2*

The effect of cover cropping varied also at the species level, as shown for Fabaceae (Fig. 5.5; Supplementary Material S5.3.3: Model 3), with the tropical legumes *Lablab purpureus* and *Mucuna pruriens* resulting in the greatest yield increases. *Lupinus* sp. performed



intermediately among the Fabaceae, but the yield increases were not significant.

Figure 5.5 *Change in main crop yield and shoot biomass after different Fabaceae cover crop genera and species. The points represent the percentage change of the modeled median (+/- 95 % CI), relative to the respective controls. On the left are displayed the number of observations. The letters indicate significant differences among cover crop species with a Tukey post-hoc test (p<0.05). Species with only one or two observations were aggregated as "other Fabaceae":* Anthyllis vulneraria, Tephrosia purpurea, Stylosanthes guianensis, Pueraria phaseoloides, Psophocarpus tetragonolobus, Mucuna cochinchinensis, Dolichos lablab, Cassia tora, Canavalia ensiformis, Cajanus cajan, Trifolium *sp.*, *and* Arachis hypogaea*. The corresponding model can be found in Supplementary Material S5.3.3*

8 Soil biological variables

To understand the mechanisms by which cover crops might stimulate P cycling and yield of the main crop, we explored the soil microbial community after application of different cover crop types. The effects on abundances of AMF, P_{mic}, and on extracellular P-cycling enzymes (phosphatases) were tested (Fig. 5.6, Supplementary Material S5.3.5: Models 5.1-5.8). Datasets for the soil biological variables included data from seven studies with 60 observations for mycorrhizal abundance, four studies with 53 observations for phosphatase activity, and two studies with 30 observations for P_{mic}. Abundance of AMF spores and root colonization increased after mycorrhizal cover crops (cover crop mixtures, Fabaceae and Poaceae), but did not change or increased only slightly after non-mycorrhizal cover crops (Brassicaceae and *Lupinus* sp.). Tillage did not significantly decrease mycorrhizal abundance in the present dataset. Cover cropping generally increased P_{mic} significantly; with Poaceae, Fabaceae, and *Lupinus* sp. resulting in the greatest increases, around 25 %, but only the effect of Poaceae was significant. Microbial biomass P showed no relationship with main crop yield or P uptake (data not shown). Extracellular phosphatase activity increased around 20 % after cover cropping, with Brassicaceae treatments tending to result in the smallest increases over the control, and with Fabaceae, lupins, and Poaceae having the largest effect. Phosphatase activity did not affect main crop growth performance (data not shown).

Standard soil P testing (i.e. Olsen P or similar) was conducted in many studies after cover cropping in order to predict P availability to the main crop. Overall, cover crops had minor effects on the pools measured with these methods, with the exception of *Lupinus* sp., which increased P_a markedly. There was no evident relationship between P_a after cover cropping and main crop yield or P uptake.

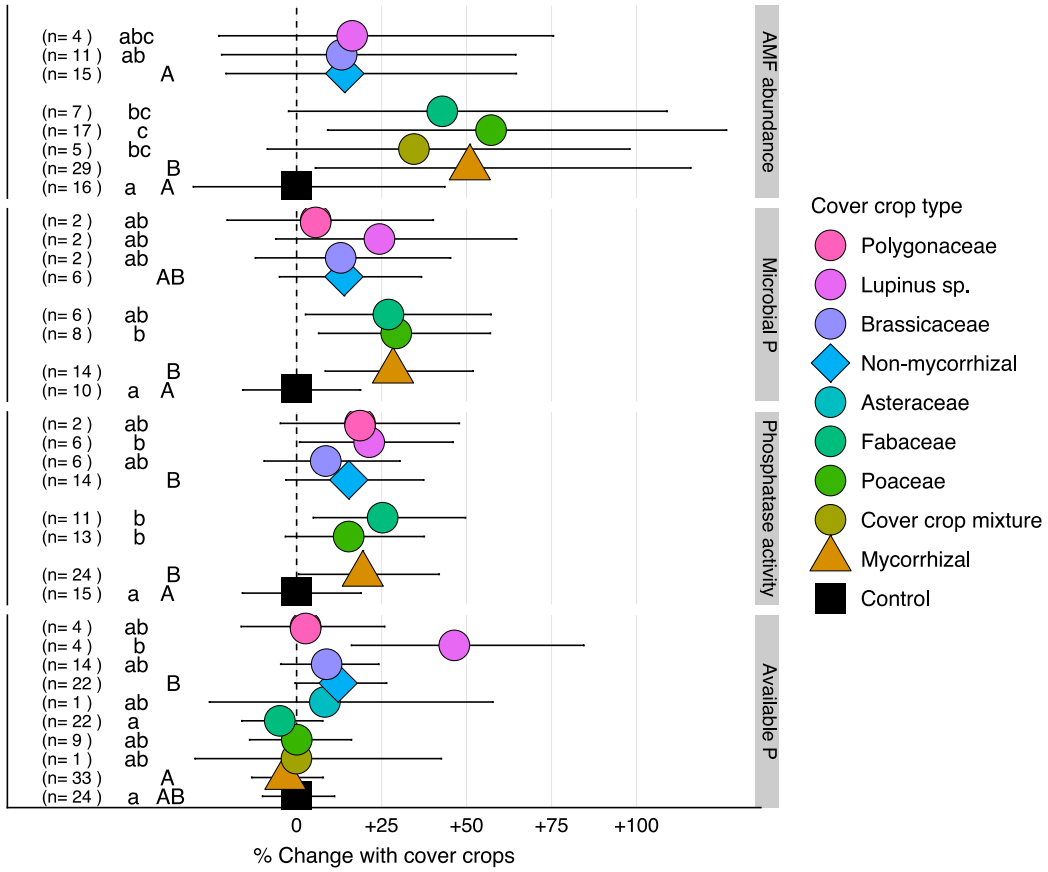


Figure 5.6 *Soil biological parameters: change in percent abundance of arbuscular mycorrhizal fungi (AMF), microbial biomass phosphorus (P) content, and phosphatase activity as well as available P after different cover crops, relative to the respective controls. On the left are displayed the number of observations. The lower-case letters indicate, for a single main crop type with a Tukey post-hoc test ($p<0.05$), significant differences among cover crop types (including the control), and the upper-case letters between mycorrhizal cover crops, nonmycorrhizal cover crops and the controls. The corresponding models can be found in Supplementary Material S5.3.5*

9 Cover crop effects under different soil P conditions

We intended to explore the effect of cover cropping on soils differing in soil P status by classifying soil P_a into *low* and *high*. The majority of studies were conducted in soils with a low P_a. Cover crops had more pronounced effects on main crop performance in these soils compared with systems with abundant labile P (Fig. 5.7; Supplementary Material S5.3.6: Models 6.1 and 6.2). Additionally, under conditions of low P_a, the cover crop benefit was greatest. Cover crop effects on soil microbial parameters were also influenced by soil P

status, reflected in a much stronger increase in AMF abundance in soils low in P_a compared with high-P_a soils (Fig. 5.8; Supplementary Material S5.3.7: Model 7).

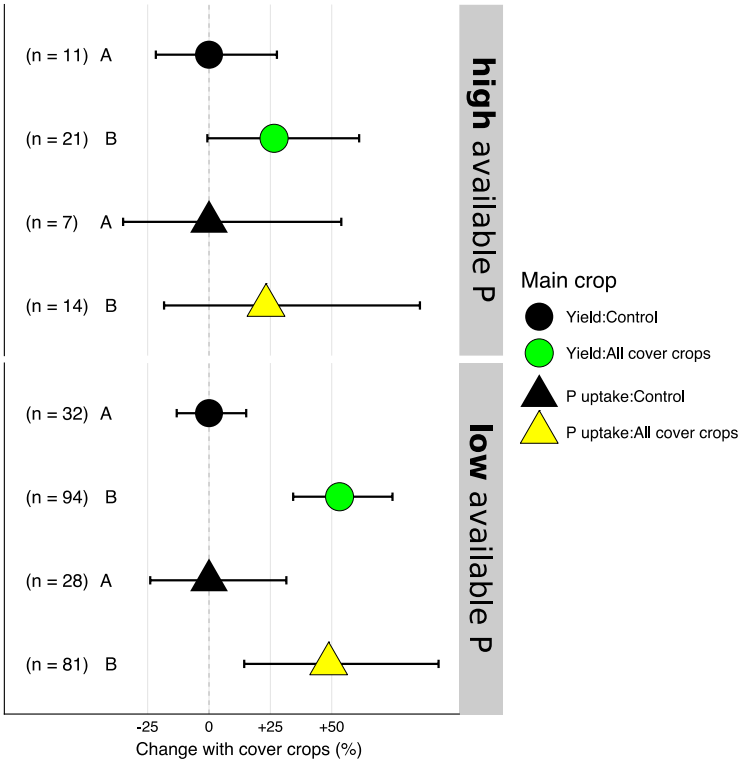


Figure 5.7 *Main crop yield and phosphorus (P) uptake as affected by cover cropping in soils with low and high available P (P_a). The points represent the percentage change of the modeled median (+/- 95 % CI) of the cover crop treatments relative to the controls without cover crops. On the left are displayed the number of observations. The letters indicate significant differences among groups with a Tukey post-hoc test ($p<0.05$). The corresponding models are presented in Supplementary Material S5.3.6*

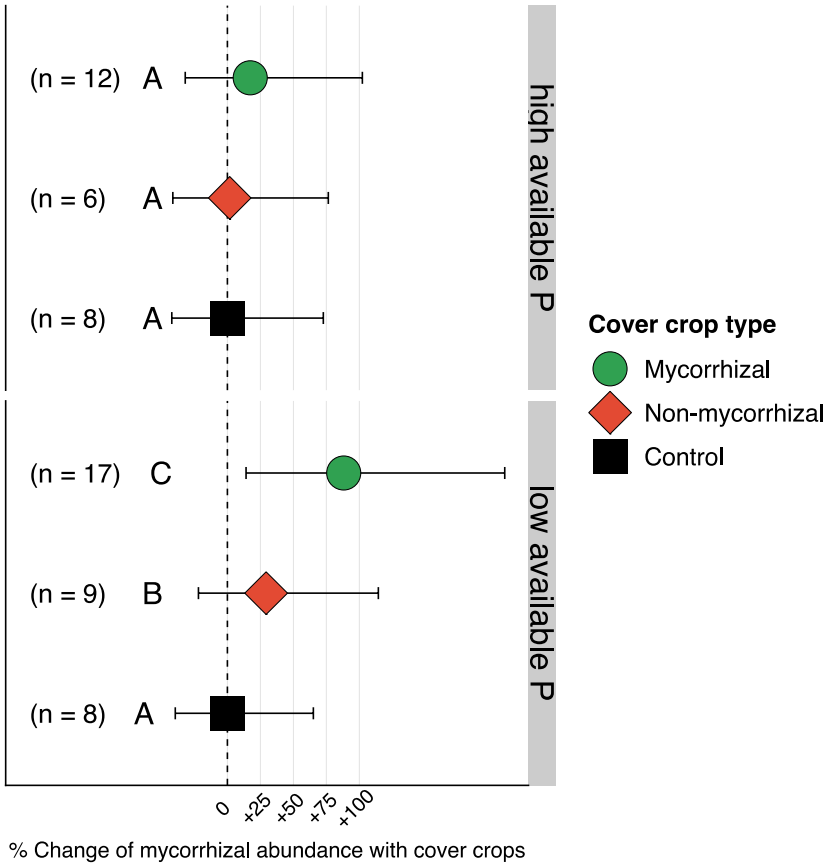


Figure 5.8 *Effect of cover crops on arbuscular mycorrhizal fungi (AMF) in soils with different P availability. The points represent the percentage change of the modeled median (+/- 95 % CI) of the cover crop treatments relative to the controls without cover crops. On the left are displayed the number of observations. The letters indicate significant differences among groups with a Tukey post-hoc test (p<0.05). The corresponding models are presented in Supplementary Material S5.3.7*

5.5 Discussion

We performed a meta-analysis to explore the importance of different plant P-acquisition strategies and to explain the benefit of cover crops on yield of main crops based on modified plant-microbe interactions during P cycling.

Main crops differ in their response to cover cropping

Our meta-analysis showed that cover crops have the potential to enhance both yield and P uptake of main crops in a variety of agroecosystems and under different management regimes, although the variance is very high. Main crops differ in their ability to profit from the P-benefit of cover crops, and this is related to their P-acquisition strategies. Maize and wetland rice yields increased more than soybean, cereals or

vegetables/cotton/Brassicaceae. The response of maize to other cover crop benefits, especially Fabaceae, has also been seen in other meta-analyses (Alvarez et al. 2017), but the modelled high response in the present dataset may be explained by the inclusion of several studies conducted in low-input agroecosystems. The yield response of wetland rice is not directly comparable with that of other main crops due to the practice of flooding and the fact that all studies were conducted with Fabaceae cover crops. The limited benefit of cover crops to some main crops, i.e. canola, vegetables, and cotton, can be attributed in part to the reduced number of trials and possibly to greater management challenges (i.e. cover crop residues interfering with seedbed preparation) compared with other, more robust, arable crops.

10 Mechanisms underpinning the P benefit of cover-crop families

The main crop response was related to both the cover crop species used and the varying mechanisms of plant-microbial interactions. Fabaceae was overall the most effective cover crop family across all conditions and systems (Figs 7.4 and 7.6). This family combines several of the mechanisms of P benefit: P uptake and carry-over in an abundant biomass with a high P concentration that facilitates release in synchrony with the main crop (Fig. 5.3), and a lasting effect on the soil microbial community (especially for mycorrhizal abundance and phosphatase activity) (Fig. 5.6). The N provided by symbiotic fixation provides an additional advantage through the acceleration of residue mineralization.

The separation of *Lupinus* sp. into a group distinct from other Fabaceae is justified: while P_{mic} and phosphatase activity were similar, the other (mycorrhizal) Fabaceae were clearly more favorable to mycorrhizal fungi than *Lupinus* sp. The most striking difference, however, was the effect on the pool of P_a (Fig. 5.6). The biochemical modification of the rhizosphere of *Lupinus* sp. increased the abundance of labile P under the main crop also, but its low biomass and low to intermediate P concentration probably limited its benefit to the main crops.

The absence of increases in main crop yield or at least P_a with pure stands of Polygonaceae, mainly *Fagopyrum esculentum* (buckwheat), was not expected in our meta-analysis, as buckwheat is used as a P-mobilizing species (Boglaienko et al. 2014). Whether or

not buckwheat's potential could be improved with other main crops, in a mixture with other cover crop species, or if its beneficial effects were limited by the low biomass observed in the studies included in the meta-analysis, warrants further investigation.

Asteraceae had intermediate positive effects on the main crops, but were tested exclusively as a cover crop for cereals. The species used in the experiments are all potentially mycorrhizal (Wang and Qiu 2006), but we lack detailed data on the soil biological parameters. The high biomass produced by these species is favorable as long as P concentrations are not too low.

Pure stands of Brassicaceae did not improve P nutrition of the main crop as much as most other families. This was possibly connected to a rather low interaction with the soil microbial community: neither mycorrhizal abundance, nor P_{mic} or phosphatase activity were increased significantly under the main crop. For species with high biomass production, such as Brassicaceae and Poaceae, P-cycling via the residue pathway is more important than for other cover crops.

Despite large amounts of P cycled through the biomass of Poaceae cover crops (Fig. 5.3), yield benefits for the main crops were limited (Fig. 5.4). Poaceae were most successful in increasing mycorrhizal abundance and microbial P, and they also enhanced phosphatase activity; negative effects on P-related soil biological parameters could therefore be ruled out. Poaceae produced the greatest quantities of biomass, but had the lowest mean P concentration of the cover crops, averaging 2 g P kg^{-1}.

11 Decomposition of cover crop residues

In spite of their high biomass production and positive effects on soil microbial properties, pure stands of Poaceae were among the least successful cover crop families regarding P benefits for the main crops. This was possibly connected to P immobilization (Eichler-Löbermann et al. 2008), but may have involved other mechanisms as well, as, e.g., incomplete termination or problems with seedbed preparation.

Cover crop biomass and P concentration determine the amount of P cycled through the biomass, which can range between 1 and 30 kg P ha^{-1}, although 3 to 10 kg P ha^{-1} is more typical, depending on cover crop species and P-availability (Fig. 5.3). The root:shoot

partitioning of P in cover crops is variable, with 16 to 65 % of the total plant P in the roots (Franchini et al. 2004). The threshold concentration of P in residues that determines immobilization/mineralization is 2-3 g P kg^{-1}. The P contained in plant residues can be divided into available P_i and a recalcitrant P_{org} pool. The chemical composition of the plant parts changes with the developmental stage of the plant and with P availability. Cover crop residues are different from harvest residues, as cover crop plants do not reach maturity and P_i is the major pool in the cells (~70 %) (Damon et al. 2014).

With tillage, 70-80 % of the P in legume cover crop residues is released after six months, with roots being slightly more recalcitrant (Talgre et al. 2012). The processes responsible for the decomposition of cover crop residues are related to the mineralization of other pools of P_{org} in the soil. Cover crops increase phosphatase activity in the soil under a main crop. Although Poaceae, Fabaceae, and lupins tended to increase enzymatic activity more than Brassicaceae did, the plant type seems less important than the practice of cover cropping itself (Fig. 5.6).

In soils with sufficient P availability, microbial P mineralization is not driven by microbial P requirements, but rather by release of plant-available P as a by-product of carbon mineralization (Spohn and Kuzyakov 2013b). Current modeling approaches assume that microbial biomass releases P upon death, and is connected to the decay of the residue biomass through the availability of C substrates. A single pool for P_{mic} and residue P_{org} is used, assuming the same decay coefficients (Damon et al. 2014; Varela et al. 2017). Some authors argue that many studies on the decomposition of cover crop residues have used unrealistically large quantities of finely-ground residues and have not taken into account modification of the rhizosphere by the cover crops (Cavigelli and Thien 2003). Additionally, high concentrations of decomposing legume residues transiently increase the pH in soils with low pH and SOM, potentially leading to increased P availability (Vanzolini et al. 2017). Further field studies on residue decomposition dynamics with tight sampling frequencies are necessary. Different experimental set-ups can shed light on the relative importance of the transfer of P via the (shoot) cover crop biomass, e.g., removing the cover crop shoots, or by applying cover crop residues to previously unplanted soil (Rutunga et al. 2008; White and Weil 2010; Buyer et al. 2010).

The mechanisms by which the soil microbial community determines P dynamics during crop residue decomposition are not fully understood (Maltais-Landry and Frossard 2015), nor are the interactions between the microbial community and particular cover crops with their subsequent decomposition dynamics. The decomposer community adjusts to the cover crop species, as decomposable plant residues are produced over the entire growing period by senesced leaves or dead root hairs. Together with root exudates, this constant input of substrates constitutes a driver for shifts in microbial community structure, increasing the numbers of fast-growing copiotrophic microbes. Due to the strong impact of nutrient availability, phylogenetic diversity decreases in the vicinity of plant roots (Marilley and Aragno 1999); nevertheless, overall species richness in the field is expected to increase due to increased spatial heterogeneity. In a litterbag study in Brazil, mixtures containing *Raphanus sativus* with *Secale cereale* or *Avena sativa* showed peculiar dynamics, with a delayed increase of P_{mic} associated with RNA that could not be explained by the chemical characteristics of the residues alone (Oliveira et al. 2017). The soil microbial community also influences suppression of weed germination by cover crop residues through selective decomposition of phytotoxic compounds (Moonen and Bàrberi 2006). The positive effects of cover crops on soil fauna (Blanchart et al. 2006) increase decomposition rates and nutrient cycling, probably persisting into the main crop phase. Although we focused on the soil microbial community, the importance of soil fauna (i.e. earthworms) for the shifts in P dynamics after cover crops should not be underestimated (Roarty et al. 2017).

The relationship between nutrient stoichiometry of the soil, microbial biomass, and plant residues determines microbial colonization and mineralization patterns. Fungi and other microorganisms capable of filamentous growth, unlike unicellular life forms, are capable of translocating nutrients between different compartments (i.e. soil-litter) to compensate for nutrient limitations. This has been demonstrated for C and N (Frey et al. 2003), and seems also plausible for P. Therefore, and bearing in mind the reduced damage to hyphae by reduced soil disturbance, fungal-driven decomposition probably dominates in no-till systems, where residues have less direct contact with the soil. Although no-till significantly increases fungal abundance, the interaction between the factors tillage and cover crop had

no significant effect on the ratio of saprotrophic fungi:total bacteria in a long-term experiment under continuous cotton in Tennessee, USA (Mbuthia et al. 2015).

Nutrients other than P also need to be taken into account (Weerakoon et al. 1992), as residue mineralization dynamics and P release can be driven by N availability. An increased supply of N from a leguminous cover crop may permit the main crop to exploit its P-acquisition potential, resulting in increased P uptake. In field experiments, this effect is difficult to control, especially when a significant proportion of nutrients is contained in the cover crop root biomass. Cover crops can cycle substantial amounts of nutrients (potassium (K), magnesium (Mg), and calcium (Ca)) in their biomass (Wendling et al. 2016), and increase the availability of K (Cardoso et al. 2013). Descriptions of negative effects on plant nutrition are scarce and remain hypothetical. In some situations, the *biomass pathway* described in this paper could increase availability of potentially toxic elements, e.g., manganese (Mn), and lead to growth depression (Horst et al. 2001). Other researchers have considered the possibility that high levels of $NaHCO_3$-P_i near the surface could induce copper (Cu) and zinc (Zn) deficiencies in conservation agriculture systems (Dube et al. 2014).

12 Arbuscular mycorrhizal fungi: early colonization assists crop P uptake by soil exploration

The strong increase, around 50 %, in AMF abundance after mycorrhizal cover crops (Fig. 5.6), is important for the mechanisms of P benefit to the main crop. Most crops, with some notable exceptions, i.e. Brassicaceae, Polygonaceae, and *Lupinus* sp., can form symbioses with AMF with multiple benefits (Koide and Mosse 2004). AMF hyphae provide some of the functions of root hairs, especially in plant species with thick roots and very few or short root hairs, leading to exploration by plants of an increased volume of soil (Smith et al. 2011). Some functions and mechanisms of AMF symbioses are well known, although several fundamental issues remain unanswered. In AMF-colonized plants, the fungi are usually involved in P uptake with inhibition of a direct pathway via roots, and there may not always be positive growth responses (Smith et al. 2015; Ryan and Graham 2018).

some agroecosystems There is no conclusive evidence that AMF-colonized plants are able to take P from soil sources that cannot be accessed by the roots themselves; rather

they increase the soil volume from which the same P pools can be acquired (Smith et al. 2015). Direct release of phosphatases by AMF with a significant contribution to plant P uptake is under discussion (Joner et al. 2000). However, the substantial C input from plants through mycorrhizal hyphae extends our concept of a modified rhizosphere to a much greater soil volume, and the microflora of the mycorrhizosphere may play a critical role in P acquisition (Bending et al. 2006).

Mycorrhizal cover crops tended to increase main crop yield and P uptake more than non-mycorrhizal plant species did (Fig. 5.4), and AMF abundance is positively related to main crop yield and P uptake (S3.9 Models 9.1 and 9.2). With phosphatase activity, this direct relationship was not found. However, methodological deficiencies and the small number of studies may have resulted in a high variance. Due to sorption and stabilization onto soil particles, the enzymatic activity of a soil also reflects the recent history of a soil. This must be taken into account when interpreting the results of the meta-analysis. Samples were taken after termination of the cover crop or under the main crop. Therefore, the enhanced activity relative to that of the fallow control treatments corresponded either to residual phosphatases released by the cover crop roots, to changes in abundance or structure of the soil microbial community, or to an overall substrate-driven increase in phosphatase activity due to P-rich cover crop residues in the soil. Increases in phosphatase activity mirrored the effect of cover crops on microbial biomass P, indicating a potential microbial origin of the enzymes.

A mycorrhizal cover crop can transfer its ability to access P in the soil to the main crop in the form of mycorrhizal inoculum (hyphae or spores in the soil). Although the AMF-plant symbiosis is unspecific and larger plants may be simultaneously colonized by different mycorrhizal fungal species, there are some plant-AMF genotype combinations that are more efficient than others (Jansa et al. 2011). Molecular techniques make it possible to describe AMF diversity associated with specific cover crops (Sharrock et al. 2004), and increases in richness in the main crop have been reported (Ramos-Zapata et al. 2012). However, current knowledge gaps regarding the connection between AMF community assemblage and species function constrains the effective translation of this information into specific cover crop species recommendations.

The build-up of AMF inoculation potential benefits only AMF-competent main crops, and the ability of the main crop to take advantage of earlier increased mycorrhization by previous cover crops determines the P benefit (Bittman et al. 2006). For maize, a positive relationship between mycorrhizal colonization and plant biomass or P content was found in both our aggregated dataset and several single studies (White and Weil 2010; Njeru et al. 2014). Cavigelli and Thien (2003) reported *Lupinus albus* unexpectedly decreased sorghum P uptake in a pot experiment, although P uptake and biomass was the highest of the tested winter cover crop species; a possible explanation was that lupin was the only non-mycorrhizal crop in the study. However, the lower potential for AMF inoculation cannot have been the only reason, as non-mycorrhizal Brassicaceae performed better, whereas mycorrhizal Poaceae combined poorly.

It is important to bear in mind that non-mycorrhizal crops have evolved special strategies for P acquisition. Non-mycorrhizal families can be broadly classified into Brassicaceae and Proteaceae groups, which evolved in P-rich and severely P-impoverished environments, respectively (Lambers and Teste 2013). *Raphanus sativus* var. *oleiferus* (oilseed radish) exudes large amounts of acid phosphatase and other rhizodeposits into the rhizosphere (Kunze et al. 2011); the exceptional P-mining strategies of *Lupinus* sp. have been described above. The impact of these P-acquisition strategies must be considered when designing site-specific crop rotations that include cover crops.

The meta-analysis also showed an unexpected slight tendency toward increased mycorrhizal abundances, after non-mycorrhizal cover crops. However, species of plant families labeled as “non-mycorrhizal” can be infected at low levels by AMF (Lambers and Teste 2013). Another possibility is that higher herbicide usage in the control treatments decreased AMF abundance because of direct toxic effects (Trappe et al. 1984; Giovannetti et al. 2006) or by fewer weeds acting as mycorrhizal hosts (Oehl et al. 2003).

The use of cover crops to build up the inoculum potential of beneficial microorganisms, including AMF, has the capacity to considerably improve soil fertility (Galvez et al. 1995; Boswell et al. 1998; Bagayoko et al. 2000; Kabir and Koide 2002; Lehman et al. 2012), although apparently not in all agroecosystems (Sorensen et al. 2005; Higo et al. 2014). In a study in USA, 31 years of *Vicia villosa* cover cropping decreased mycorrhizal abundance

relative to wheat or no cover crop, with some responses associated with high N rates (Mbuthia et al. 2015). Also, in fields with a history of mycorrhiza-enhancing cropping techniques (i.e. rotation dominated by mycorrhizal crops, no-till), cover crops may not increase the mycorrhization of the main crop further (Turmel et al. 2011). In agricultural soils very low in mycorrhizal abundance, cover crops may fail to increase the inoculum potential above a minimum threshold necessary to benefit plant growth (Douds et al. 2011). On the other hand, cover crop mixtures and a strategic AMF-build-up may be especially important in this context (Lehman et al. 2012).

Weeds growing during the off-season may also result in benefits for the main crop; e.g., *Taraxacum officinale* (dandelion) is a good host for overwintering mycorrhizal fungi (Kabir and Koide 2000). However, some weeds induce negative changes in the microbial community (i.e. a decrease in AMF) and enhance their own competitive advantage over the crops (Wortman et al. 2013). Costs of seeds and labor for cover crop establishment have to be included in evaluating their potential to outperform weeds, and, above all, their easy termination (Wang et al. 2008). Also, management and application form of cover crops determines their effect on AMF, as fresh red clover residues, directly incorporated or used as a mulch layer, result in greater abundance of AMF compared with processed residues (biogas slurry, compost) (Elfstrand et al. 2007). The discovery of significant advantages to seedlings conferred by early establishment of symbioses with mycorrhizal fungi or P-solubilizing bacteria has prompted the development of commercial and on-farm produced inocula for plant growth promotion (Douds et al. 2010). Also, combining cover crops with a simultaneous inoculation of microorganisms has been investigated (Cui et al. 2015).

It is experimentally challenging to separate the direct benefits of improved P availability from other plant-microbial interactions and indirect plant growth-promoting effects. Microbes have multiple effects on plant health via plant pathogen suppression as well as on nutrient cycling and plant nutrition (Bagayoko et al. 2000; Bashan et al. 2013). Cover crop species differ substantially in their root-associated fungal communities (Benitez et al. 2016), suggesting opportunities for management of beneficial and pathogenic fungi, although contradictory evidence has been reported (Turrini et al. 2016). Nutrient uptake and crop yield are often limited by biotic stress. Increased aboveground biodiversity (i.e. by cover

cropping) can suppress soil pathogens, thus promoting plant growth. Some cover crop species can be used for specific pest management, whereas others may serve as hosts for pests (Ratnadass et al. 2012). In view of the multiple ecosystem functions of soil microbes, Festers & Sawers (2011) advocated a holistic approach of increasing biodiversity through agronomic management as opposed to a reductionist approach focusing on single species.

13 Microbial P as a significant pool

Increases in microbial P of around 25 % with Poaceae and Fabaceae cover crops (Fig. 5.6) are worth further discussion, because P_{mic} constitutes an important pool in soil due to its relatively fast turnover and subsequent availability for plants. However, due to the small number of studies, we did not detect a significant effect of P_{mic} on main crop yield and P uptake. Interestingly, the mycorrhizal cover crop types tended to enhance P_{mic} more than the non-mycorrhizal species. The pool of P_{mic} in agricultural soils typically constitutes 5–70 kg P ha^{-1}, with turnover times of a few months, depending on management and C inputs (Oehl et al. 2001).

Phosphorus in microbial cells is present mostly in the form of nucleic acids, but also as small P-containing esters, free P_i, and phospholipids of cell membranes. The nucleotide content can vary greatly, depending on the growth rate of the cell; surplus P can be stored as polyphosphates (Harold 1966; Stewart and Tiessen 1987). When comparing the nutrient stoichiometry of soils, microbial biomass, P_{mic} appears to be closely linked to overall microbial biomass (Cleveland and Liptzin 2007). However, in agricultural soils, the microbial C:P stoichiometry may, in response to soil fertility and management, exhibit some plasticity and be affected by the availability of P for the main crop.

Cover-cropping frequency is a greater driver of increases in microbial biomass than compost application, increasing the abundances of *Pseudomonas* and *Agromyces* species, including species that are important biological control agents and plant growth-promoting rhizobacteria (Brennan and Acosta-Martinez 2017). In a study in semi-arid Kenya, microbial C, N, and P were strongly increased by different *Brachiaria* species (Gichangi et al. 2016). In another study, cover cropping was far more important than the measured environmental variables (moisture, temperature, pH) in controlling soil microbial community structure

(Buyer et al. 2010). Relative abundance of Gram-positive bacteria was decreased by cover cropping, probably connected to their lower ability to use labile C-inputs compared with other microbial groups. In a field experiment in Sweden, direct incorporation of a red clover crop enhanced and sustained microbial biomass and soil enzyme activities more than did processed forms of green manure applied as biogas slurry or compost (Elfstrand et al. 2007).

The application of isotopic dilution methods has revealed that microbial immobilization and remineralization, rather than mineralization of non-living organic P, represents most of the gross organic P mineralization flux (Bünemann 2015). Sorption/desorption processes dominate in agricultural soils low in microbial biomass. Immobilization/mineralization dynamics are closely linked to overall microbial growth, but trophic interactions should not be overlooked, as amoebae and other bacteria-grazing microfauna are responsible for the remineralization of P_{mic} (Cole et al. 1977). Cover crops increase the abundance and diversity of microfauna (Blanchart et al. 2006) which restricts long-term microbial immobilization during the decomposition of plant litter. Given the close relationship between *Phaseolus vulgaris* yield and P_{mic}, microbial biomass P was proposed as an indicator of soil P availability on P-sorbing Andosols in Japan, instead of the widely used Truog-P (Sugito et al. 2010).

Mobilized and mineralized P is often intercepted by microorganisms before plant roots can take it up (Joner et al. 2000). Although in managed agroecosystems crop yield is the primary objective, some interactions between crops and other organisms, seen as competitive and undesirable in the short term, may have, in principle, longer-term favorable properties (i.e. SOM build-up, pathogen resistance). Competition for available nutrients determines the outcome of the plant-microbe relationship at all levels, from the more opportunistic microbes in the rhizosphere to the mutualists, as even AMF-symbioses can range from being highly beneficial for both partners to 'parasitic' (Johnson et al. 1997). In addition to mycorrhizal fungi, there are many other plant-associated organisms that affect P uptake. One example of the very complex interactions between microorganisms and plants are mycorrhiza-helper bacteria, which facilitate the establishment of the symbiosis (Frey-Klett et al. 2007).

14 Cover crops under different soil-management strategies

The effects of cover crops vary greatly, and some studies report no or even negative effects (Kuo et al. 2005; Takeda et al. 2009b; Rick et al. 2011). This has been due, in some cases, to intrinsic agronomic conditions related to low cover crop biomass or absence of a significant P limitation on experimental plots. Rick et al. (2011) did not find effects of cover crops on labile soil P fractions, wheat biomass, or P concentration, despite differing biomass and P concentrations of the cover crops. However, as neither cover crops nor rock phosphate fertilization resulted in substantial yield responses, it is possible that the lack of positive results was due to a combination of moderately high soil P levels, N limitation, and low precipitation. Also, studies in Brazil on strongly P-sorbing acidic soils, where cover crops often increase labile and moderately labile P pools while decreasing residual P, did not always show the expected increases in terms of P uptake (Almeida and Rosolem 2016). After three years in a Brazilian Hapludox under no-till, *Avena strigosa* (black oat), *Vicia sativa* (common vetch), and *Raphanus sativus* (fodder radish) as cover crops cycled P from the non-labile and moderately labile P pools through their biomass without reducing labile P fractions; maize yields were also not affected (Pavinato et al. 2017). Increased P availability was also not consistently translated into improved main crop performance (Pavinato et al. 2017). In other studies, however, although labile P fractions were not affected, yields were increased (Murungu et al. 2011b; Dube et al. 2014), or traditional soil P tests failed to detect the shift in P dynamics by cover crops (Takeda et al. 2009a, b). Reasons for this variability in response are manifold, and reflect both methodological limitations when assessing P dynamics and the diversity of the studies' designs, as well as abiotic and biotic factors. This points to practical problems in agricultural management that often hamper the successful exploitation of cover crop benefits. Recurrent calls for genetically-engineered crops for improved P efficiency, e.g., in Hunter et al. (2014) overestimate our understanding of the complex rhizosphere processes involved, while existing agricultural management options are underrated.

The importance of site conditions and agricultural management in controlling both growth of cover crops and main crops and the complex mechanisms of their interactions with P explains some of the variation in results of the studies included in the meta-analysis.

However, knowledge of the impact of soil P status and different management approaches will not only aid in the interpretation of field experiments, it will also provide many tools for adapting cover crop effects to the specific needs of local agroecosystems. Cover-cropping management can be used according to site conditions in many ways, e.g., by appropriate combinations of cover crop species/mixtures with the crop rotation (e.g., Oliveira et al. 2017), and by fertilization and tillage (e.g., Mbuthia et al. 2015; Teles et al. 2017). Other management decisions, such as seeding rate (Brennan et al. 2009), seeding and termination date (Nascente et al. 2013), and termination technique (Dorn et al. 2013), extend the opportunities for fine-tuning cover crop performance and P effect. However, as cover crops provide multi-functional tools when enhancing performance for a given function, there can be trade-offs with other functions (Mirsky et al. 2012). For example, on soils with low P availability, a highly efficient scavenging cover crop with an extensive rooting system that takes up P from the same pools as the main crop could lead to soil P immobilization when the easily-available P is depleted and immobilized in recalcitrant plant residues. Management options could include selection of a different cover crop species/mixture or a later sowing/earlier termination date to reduce the recalcitrance of cover crop residues.

15 Soil P and fertilization

Phosphorus dynamics, and the effect of cover crops on them, are strongly influenced by the soil, specifically by the size of easily- and sparingly-available P pools. The meta-analysis supports the hypothesis that cover crop P benefits are more evident in soils poor in P_a than on sites with higher P_a (Fig. 5.7). Also, interactions between cover crops and the soil microbial community are influenced by soil P status: the increase in AMF abundance after mycorrhizal cover crop use is much greater in soils low in P_a (Fig. 5.1); phosphatase activity shows a similar pattern (data not shown). These results are promising, and the P-mobilizing properties of different cover crop species should be further investigated, specifically in soils high in P_t, but low in P_a. However, soils very low in P_a often also have other fertility problems. Soil quality affects the beneficial effects of cover crops in several ways. Although very fertile or well-managed soils are difficult to improve further with cover crops (Turmel et al. 2011), cover crops require adequate initial soil fertility to effectively improve P cycling and inputs may be necessary to obtain a functional cover crop (Jensen et al. 2005).

Examples of useful soil quality parameters are available P, microbial biomass C (C_{mic}), and the C_{mic}:C ratio (Koné et al. 2008). Depending on local economic circumstances, a good use of expensive inorganic P fertilizer might be to apply P sources to cover crop legumes; this would improve their ability to benefit from existing soil N as well as residually applied P (Carsky et al. 2001). There are substantial synergies between P application and N fixation by leguminous cover crops (Weerakoon et al. 1992).

Several studies that did not find a consistent effect of cover crops on soil P pools led some authors to argue that the P-mobilizing properties of legumes are more efficient for low-input agriculture on soils with low P availability than for systems where P availability is higher and P limitation weaker (Maltais-Landry et al. 2015). We agree with this line of reasoning and suggest consideration of P reserves to complement the labile P assessed in standard soil P testing when selecting and evaluating cover crops. The classification into high- and low-P soils is a simplification, which becomes clear when treating soils high in P_t but very low in P_a, as in southern China or Andosols in Chile and Japan. On these soils, P-mobilizing cover crops would be of particular interest. Because cover crop P benefit reflects mainly biological processes, these may not be assessed properly with methods designed to predict the effects of inorganic P fertilization. In high-input systems with high P availability, tillage, and additional P fertilization, cover crops often do not increase yields significantly over the control (data not shown).

A positive interaction of cover crops with P fertilization is in part related to the fertilizer type used. Cover crops may increase the availability of the P contained in organic fertilizers or phosphate rock (Ca-P/apatite). In particular, proton- and organic anion-exuding plants such as *Fagopyrum esculentum* or legumes, but also green manure from *Tithonia diversifolia*, dissolve calcium (Ca)-P, which is important for organic farmers on calcareous soils (Arcand et al. 2010) and farmers relying on phosphate rock on acid soils (Somado et al. 2003; Ikerra et al. 2006; Oikeh et al. 2008; Opala et al. 2010). However, this effect has not always been found (Rick et al. 2011). Plant-microbial interactions likely play a role in fertilizer effects (Bah et al. 2006). Indications of an interaction between P-fertilizer type (soluble P vs rock phosphate) and cover cropping has also been shown in some studies (Almeida and Rosolem 2016), but was not considered in the present meta-analysis. Plant

species respond differently to fertilizer types. *Brassica oleracea* showed a dramatic response to an otherwise unreactive, Fe-rich, igneous phosphate rock, but this source was ineffective for leguminous cover crops and maize; the following maize yield was not increased by cabbage (Weil 2000). Aluminium phosphate is naturally present in some rock phosphates and soils, but may be inadequate for plants that rely on proton exudation for P-acquisition, as pointed out by Pearse et al. (2006), comparing wheat and lupin in a pot experiment. The interactions between fertilizer type, cropping system and soil are complex (Romanyà and Rovira 2009), and require, therefore, site-specific recommendations.

Some studies have shown a more efficient use of P when added with organic amendments (Eichler-Löbermann et al. 2008; Maltais-Landry et al. 2015), but not others (Takeda et al. 2009a). Under the condition of added C sources, effects of direct competition for P_a by microbial immobilization are at variance with a rate increase in cycling of organic P. The properties of the amendment must be taken into account when selecting the cover crop. Fertilizers with high levels of P_a should be combined with species such as *Raphanus sativus oleiformis* (oil radish) that maximize uptake and biomass production; species with mobilization traits, such as phacelia would be suitable for sites with less-available P sources (Bachmann and Eichler-Löbermann 2010).

Continuous developments in alternative and innovative cover-crop systems have the potential to increase sustainability of intensive systems such as high-input horticulture (Brennan 2017). In systems with substantial organic inputs (i.e. manure), P availability is usually not a concern, and other cover crop effects are desired (prevention of nutrient leaching, weed suppression, C input). An overlooked property of cover crops regarding P dynamics is connected to the inherent imbalance of the N:P ratio in animal manure: legume cover cropping permits lower rates of manure application by supplying N to subsequent crops (i.e. supporting P-based application rates), reducing the P excess of N-based organic fertilization (Kleinman et al. 2001; Cherr et al. 2006). A special, but not uncommon, situation is the management of large quantities of manure from industrial animal farms by field application in combination with cover crops (Rowe et al. 2006).

Cover crops can be used in watershed management for the reduction of P-runoff (Villamil et al. 2006; Geleta et al. 2006). Legumes are generally appreciated for their ability to

mobilize poorly-available P, whereas grasses are more often used as “catch crops”, scavenging the available nutrients and reducing losses (Maltais-Landry et al. 2015). The authors concluded that, in soils with low P-sorbing capacity, P transfer via the cover crop biomass of grasses is more effective than that of legumes, which more strongly modify their rhizosphere. Systems at risk of P losses usually also have problems with N losses (Sharpley and Smith 1991; Aronsson et al. 2016; Brennan 2017), making a focus on grasses instead of legumes meaningful.

16 Cover crop mixtures

Cover crop mixtures are, in terms of main crop performance, superior to monoculture cover crop species (Figs 5.4-5.6); however, most studies used maize as a main crop, which is highly responsive to cover cropping, and also the total number of trials was low. It is difficult, therefore, to draw general conclusions about cover crop mixtures due to the inherent differences in systems depending on the specific components. Mixtures frequently outperform single species in terms of biomass production and P uptake (Li et al. 2007, 2014; Messiga et al. 2016), in addition to the positive influence plant biodiversity exerts on soil biology. Other plants growing in close association with P-mobilizing plants confer additional benefits through intercropping or undersowing, and may also increase access to sparingly-soluble P (Li et al. 2007). Cereal-legume mixtures are among the most widely used and studied (Tarui et al. 2013), due to both their ecological importance and to the availability of practical management expertise with these combinations, because of their use in fodder production. An important benefit of this association is increased N-fixation by the legume driven by the N-demand of the cereal (Høgh-Jensen and Schjørring 1997), N transfer (Brophy et al. 1987), and facilitative interactions via root exudates, as detected between *Zea maize* and *Vicia faba* (Li et al. 2016). In cereal-legume mixtures, the cereal component benefited more from the intercropping association than the legumes, reflected in the observed shift in composition towards a higher proportion of grasses over time (Maltais-Landry 2015). However, the legume productivity may also be enhanced by intercropping, because *Zea maize* improved Fe nutrition of *Arachis hypogaea* (peanut) in a calcareous soil (Zuo et al. 2000; Zuo and Zhang 2008). In mixtures, there are several trade-offs that have to

be balanced; e.g., easily decomposable cover crop residues improve nutrient availability for the main crop, but result in lower weed suppression and less SOM production in comparison with more recalcitrant plant residues (Tarui et al. 2013).

Desirable mixtures of species with complementary functions have a huge potential to increase the benefit of cover cropping, as, e.g., *Brassica napus* (fodder rape) and *Lupinus albus* (Little et al. 2004). There is also the advantage of the biofumigant properties of *Brassica* species and its biomass, as well as the P mobilization of *Lupinus* sp. (although a mycorrhizal component could enrich the combination). The combined use of a mixture of rye and oat as winter-hardy and winter-killed species also yielded positive results in temperate climates (Kabir and Koide 2002). The transfer of nutrients from dying roots to living roots via AMF, as suggested by Newman and Eason (1989), should also be taken into consideration.

In the meta-analysis, AMF abundance following mixtures containing non-mycorrhizal Brassicaceae tended to be only slightly less than that with pure Poaceae or Fabaceae stands. This suggests that one mycorrhizal partner in a mixture is capable of compensating for the lack of mycorrhizal association of the other. An experiment in mid-Atlantic USA showed that a pure stand of *Raphanus sativus* var. *longipinnatus* (forage radish) did not have a negative effect on AMF-colonization, but a pure stand of rye showed a higher rate of colonization than the mixture rye-forage radish. Further research is necessary to determine whether or not Brassicaceae inhibit the extent to which Poaceae are colonized by AMF (White and Weil 2010). As for potentially adverse effects resulting from nonmycorrhizal and mycorrhizal plant species, there are indications that members of the Proteaceae type exhibit fewer competitive interactions than Brassicaceae types (Gardner and Boundy 1983; Lambers and Teste 2013; Lambers et al. 2018).

The plethora of possible combinations and proportions in cover crop mixtures is difficult to handle experimentally. Even studies with more than four species are scarce, although a systematic and simultaneous screening would be important for the selection of appropriate mixture candidates (Horst et al. 2001; Oikeh et al. 2008; Wendling et al. 2016). The selection and testing of innovative cover crop species requires more large-scale projects, such as OSCAR (Crossland et al. 2015), or the 2017 started EU-project REMIX. There is substantial

variation, even among genotypes, regarding P-uptake efficiency and subsequent main crop growth (Jemo et al. 2006; Rose et al. 2010a; Pang et al. 2018b). Likewise, site conditions are important, because in an acidic soil, root growth determined P uptake, whereas in an alkaline soil malate exudation was probably the more important parameter (Rose et al. 2010a). With respect to soil biological properties, a truly novel and strategic experimental approach is necessary to assess the multiple combinations of cover crop mixtures.

The systematic use of plant traits that interact with P dynamics, as proposed by Wendling et al. (2016), is already used for other cover crop functions (Damour et al. 2014), and constitutes an important step toward increasing the comparability of trials.

17 Interactions of cover crops and tillage

Cover cropping tends to have greater positive effects on main crop performance in systems under reduced tillage/no-till than under conventional tillage (Fig. S5.6). Systems using reduced tillage benefit from the inclusion of cover crops due to weed control, nutrient release, and improved soil structure, together with synergies between both practices regarding soil biological activity. Tillage regime can vary in intensity (shallow/ non-inversion) and frequency (up to no-till), affecting, mainly in two ways, the processes by which cover crops influence soil P dynamics. First is litter distribution; tillage mixes plant residues into the soil, whereas no-till leaves the nutrient-rich residues on the top. Second, tillage regime changes the soil biota, because organisms differ in their sensitivity to soil perturbation.

Cover crops and no-till benefit soil fauna, which are important for residue decomposition, because their feeding activity fragments and buries litter, increasing the surface for microbial decay. Earthworms increase P availability and their interaction with cover crops deserves further attention (Vos et al. 2014). In tropical and arid areas, termites fulfill important functions in nutrient cycling (Rückamp 2011). On the other hand, under conventional tillage, the greater area of plant residue contact with soil increases the decomposition rate, but can lead to P sorption (Tiecher et al. 2012b). The choice of cover crop can have additional effects, e.g., with the release of isothiocyanates by tilled residues of *Brassica juncea* (Indian mustard) used as biofumigants, possibly increasing negative

effects on mycorrhizal-inoculum potential (Njeru et al. 2014). Under no-till, a forage radish cover crop did not negatively affect mycorrhization of maize (White and Weil 2010).

Balota et al. (2014) found that several microbial parameters increased under cover crops, both under no-tillage and under conventional tillage in a Brazilian oxisol. There was a greater relative increase with cover cropping under conventional tillage, although total microbial abundance and activity remained higher under no-tillage when the entire profile was taken into account. The higher metabolic efficiency (lower qCO_2–values) indicated that winter cover crops and zero tillage resulted in a more stable system. In the USA, no-till plots increased levels of Gram-positive bacteria, actinomycetes, AMF, and enzymatic activity, whereas tillage enhanced the abundance of saprotrophic fungi and provided a greater total microbial biomass. The cotton yield was greatest after *Vicia villosa* under no-till (Mbuthia et al. 2015).

One of the principal reasons for the inclusion of cover crops in temperate agroecosystems is the alleviation of soil compaction, which in turn expands the effective rooting zone, benefiting main crops possessing weaker root systems (Calonego and Rosolem 2010). In this context, the property of *Raphanus sativus*, the so called “tillage radish”, deserves further attention, as it produces a strong taproot that decays during the winter and leaves distinct biopores in the surface soil, potentially leading to locally greater P-availability (White and Weil 2011).

Cover crop residues at the soil surface can delay soil warming in spring, and, therefore, the onset of microbial activity; this constrains the use of mulches in cold climates (Sarrantonio and Gallandt 2003). The increased soil water content under mulch layers can also constitute a problem in wet years (White and Weil 2010). Some studies suggest that cover crops have only limited potential to decrease P losses under the wet and cold climatic conditions of northern Europe, as dissolved organic P leaching over winter may outweigh reductions in erosion losses when plant residues experience several freeze-thaw cycles in the field (Bergström et al. 2015; Aronsson et al. 2016; Kirchmann and Wessling 2017). However, cover crops that were incorporated into the soil during winter did not increase P leaching in a study in Belgium (Vanden Nest et al. 2014); similarly, at least in milder Nordic climates, winter-hardy cover crops could be an option.

18 The termination method

When cover crops do not die off via natural causes (e.g., frost or drought), a termination step is required. The most widespread method is a termination herbicide, usually glyphosate. However, this practice has been criticized because of undesirable side effects on the agroecosystem and the environment (Johal and Huber 2009; Yamada et al. 2009; Mamy et al. 2016). Glyphosate may also reduce main crop yields when applied too close to the main crop seeding date (Nascente et al. 2013) or it may interact with P-fertilizer application (Rose et al. 2018). Termination can also be mechanical, by tillage of varying degrees of intensity, mixing the plant residues with the soil, or by flailing, disking or rolling the shoot biomass, resulting in a mulch layer that covers the soil surface. The use of roll-choppers requires exact timing to be effective, but can be an adequate alternative to glyphosate (Creamer and Dabney 2002; Dorn et al. 2013). The termination step interacts with the temporal dynamics of the residue mineralization, important for synchronization of nutrient release with the requirements of the main crop (Zibilske and Makus 2009; Murungu et al. 2011a; Damon et al. 2014). The achievement of desirable C:N:P ratios with timely termination may be constrained by field accessibility related to soil water content (Odhiambo and Bomke 2007). The cover crop species should be adapted to variations in sowing and termination dates in order to achieve high biomass and nutrient content, which does not necessarily follow a linear pattern (Anugroho et al. 2009). Cover crops are usually terminated between one and a few weeks before planting the main crop in cases where they are not winter-killed. With chemical termination, a certain time for pesticide inactivation needs to be taken into account.

In some rotations, a cover crop (e.g., white clover before maize) may simply be clipped and permitted to regrow as living mulch, conferring high AMF inoculation potential and improving P nutrition (Deguchi et al. 2007). An interesting approach for integrated crop-livestock systems consists of cover crop termination by grazing (Clark 2008), which can increase P efficiency under adequate management (Costa et al. 2014). Nutrient transfer by cut and carry use of green manure cover crops on improved fallow fields may lead to nutrient impoverishment in low-input systems. Shrub species with high litter and root production, such as *Tithonia diversifolia*, may be advantageous in these situations (Rutunga

et al. 2008). Burning of bulky cover crop biomass, as practiced by some resource-limited smallholder farmers, reduces fertility benefits and increases nutrient losses (Oikeh et al. 2008).

19 Cover cropping: long-term effects and adoption

Contrary to our expectations, the published studies found no strong evidence that cover crop effects on P nutrition of the main crop increase with time after introduction into the cropping system. The few long-term studies available (Kuo et al. 2005; Abdollahi and Munkholm 2014; Mbuthia et al. 2015; Mukumbareza et al. 2015) did not report substantially better results regarding P benefit than short-term experiments over one or two years. This indicates that cover crop management and selection of appropriate species could be more important than time since adoption of cover cropping, although more long-term trials are warranted. However, all the aforementioned studies and more (Balota et al. 2014) reported increases in microbial biomass and enzymatic activity. Regarding P pools, cover crops increased mainly the P_{org} and P_{mic} pools over time (Maltais-Landry et al. 2015; Mukumbareza et al. 2015) which may have been related to overall increases in SOM (Blanco-Canqui et al. 2015). Another study found no changes in soil P fractions after nine years of cover cropping in a temperate system with high P availability (Kuo et al. 2005).

Cover cropping tends to provide better results in tropical than in temperate climates (data not shown). However, the numerous studies that show important main crop P benefits by cover cropping in temperate systems suggest that perhaps not only climate makes a difference, but also the prevailing agroecosystem. Studies in the tropics often involve low-P soils receiving fertilizer treatments, and are therefore more responsive to cover cropping compared to the northern countries whose mouldboard-ploughed fields are high in P_a. Additionally, given the management challenges of cover cropping, the greater experience with cover crops in the tropical regions may be an additional factor. However, except for regions with the limiting factors of water scarcity (as, e.g., in Mediterranean climates or with further climate change) and the short vegetation periods of cold areas, which strongly influence both plant growth and decomposition dynamics, cover cropping has the potential to be a successful management option in most climates.

Cover cropping is one of many agricultural tools and must be integrated and adapted adequately into the management strategy of an agroecosystem. Evaluation of both the multiple benefits and site-specific management needs of cover crops requires multi-faceted and whole-system approaches in research and extension (Cherr et al. 2006). Cover crop species relevant for the local farming system for other reasons than P nutrition should also be tested. Economic benefits may be indirect, e.g., from the possibility of reduced planting densities after cover crops (Wang et al. 2008). An interdisciplinary or multidisciplinary approach involving farmers and other practitioners at early stages of experimental design may increase the efficiency and practical relevance of scientific studies (Weil and Kremen 2007; Reed 2008; Scopel et al. 2013; Smith et al. 2014).

Despite its many benefits, the use of cover cropping by farmers is often less utilized than is desirable. Barriers to adoption include the following: benefits are site- and soil-specific, establishment and management problems exist, and climatic variability can lead to uncertainty in outcome (Ro et al. 2016). Water use by the cover crop may be a problem under drought stress, resulting in lower yields and P uptake (Turmel et al. 2011). Economic considerations are necessary for adoption of a farming practice: in warmer climates there may be no off-season which means that cover crops compete directly with cash crops for the same space. To compensate for the income loss, the benefit over a continuous cropping system needs to be very high. Easily-manageable multi-purpose cover crops (edible seeds, fodder, wood) would be required to increase adoption by smallholder farmers in developing countries. Also, external factors, such as changes in land markets or novel pests, determine the adoption or abandonment of sustainable practices (Neill and Lee 2001). Reasons for low adoption of cover crops in temperate high-input systems are discussed by Brennan (2017).

The yield and P effects of cover crops hold across both organic and conventional cropping systems, as the results from five studies with 45 observations using organic fertilizers and no chemical weed control were not significantly different from the nine studies with 103 observations using conventional management practices (data not shown). In order to achieve widespread adoption of cover cropping, it is essential to overcome constraints to its adoption (e.g., insufficient experience for specific site conditions, unavailability of machinery, missing management alternatives in case of cover crop failure, and general

pressure by markets to externalize costs). Improved management, increased knowledge, and development of instruments to relieve farmers of socially and environmentally unsustainable short-term market pressures, as for example community supported agriculture (CSA), are important (Lass et al. 2003). With rising fertilizer prices and pressure to reduce environmental impacts, cover crops constitute a promising, multifunctional tool for sustainable intensification of agriculture, on the conditions that species selection and management match the agricultural goals (Blanco-Canqui et al. 2015).

5.6 Conclusions

Cover crops can be successfully used to stimulate main crop yield and P uptake. However, site conditions and agronomic management lead to varying results. Plants have different P-acquisition strategies, and main crops show varying abilities to take advantage of the cover crop legacy. Cover crops benefit the P nutrition of main crops by different, simultaneous processes: soil P (sometimes from sparingly-available pools) accumulates in the cover crop biomass, and the mineralization of P-rich litter provides available P for the main crop (*plant-storage pathway*). This pathway is most relevant for cover crops with high biomass such as Poaceae, Brassicaceae, and Fabaceae (Fig. 5.9). The P concentration of the cover crop biomass determines mineralization dynamics, which may partly explain the limited efficacy of Poaceae cover crops. Cover cropping enhances soil microbial community abundance (P_{mic}) and activity (extracellular phosphatase activity), and maize, in particular, benefits from increases in AMF abundance in soils with low available P (*soil microbe pathway*). Poaceae and Fabaceae have the greatest impact on soil microorganisms. Other cover crop species (e.g., *Lupinus* sp.) are capable of mining P pools, improving soil P availability even during the main crop phase (*biochemical rhizosphere modification pathway*).

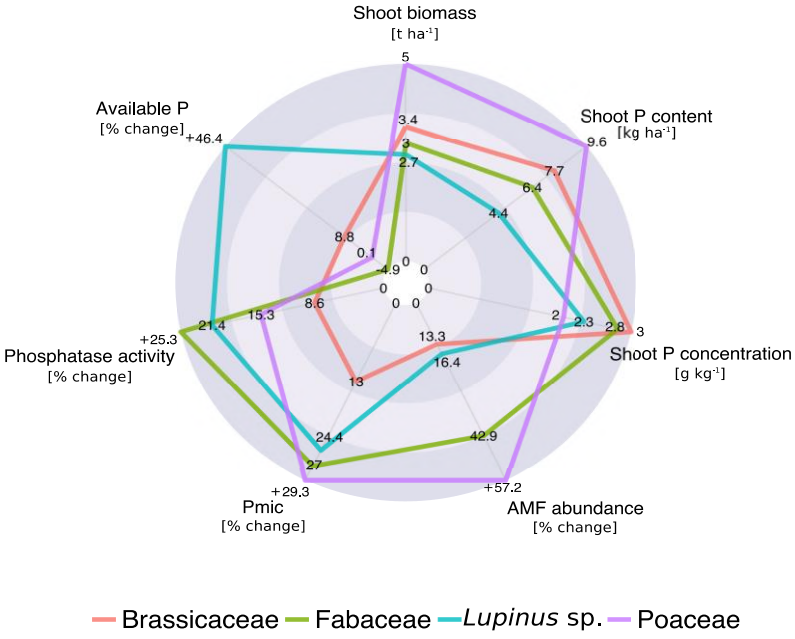


Figure 5.9 *Radar chart summarizing the properties of the cover crop families and their effects on soil. The lines correspond to the calculated quantile moment of each data point (R code provided in Supplementary Material S5.4). Grid lines correspond to the 0, 25, 50, 75, and 100-quantiles of each variable. Asteraceae and Polygonaceae had missing data points and could not be displayed here.*

Consideration of the abovementioned P-acquisition mechanisms, the interactions with the crop rotation, and the use of plant traits for the characterization of cover crop species would facilitate the generalization of the results of different studies. Further research is needed to elucidate the relative contributions of the different P-acquisition mechanisms, both to P uptake of the cover crop and their effects on the main crop, in order to optimize combinations. Soil P availability affects the P dynamics of the system and the mechanisms of cover crop benefit: generally, cover crops enhance main crop yield and P uptake in systems low in P_a more than in systems with abundant P_a, and have a greater effect on abundance of AMF.

Cover crop benefits are greater with reduced tillage or no-till. Management determines the success of cover cropping in general, and it is possible to fine-tune P dynamics with appropriate techniques. Cover crops are used on a global scale under varying circumstances with successful examples in many regions and agroecosystems. However, cover crops may

not be the optimal solution for P management in all situations. Factors that limit the use of cover crops, i.e. adverse climatic conditions (water scarcity, short growing seasons, freeze-thaw cycles), insufficient soil fertility, or problems with pests can be overcome by cover crop selection and management. However, for conventional high-input systems, though there is little likelihood of yield improvement, there is potential for environmental benefits.

Finally, in many areas of the world, the principal reason to include cover crops into the rotation is erosion control, which is probably the most important global issue regarding P management and soil in general. We need to avoid perpetuating systems in which single characteristics are overemphasized, resulting in significant trade-offs in overall performance and sustainability of agroecosystems. The isolated effect of a management practice (cover crop) on a single nutrient (P), may make sense in situations with one dominating limiting nutrient. Yet a more comprehensive evaluation of the ecosystem is required in most situations (Schipanski et al. 2014). This is especially the case for complex systems that replace technological inputs with ecosystem services of biological components. We need to find a balance that takes advantage of the numerous contributions of cover crops to agroecosystem health.

The effects of cover crops on P uptake of the main crop depend on many factors, offering opportunities for site-specific adoption and optimization of the system, but also restraining general agronomic recommendations. However, we can draw some broad conclusions about the potential for P management by cover-crop management and directions for future investigation:

- The different mechanisms of cover crop P benefit we have discussed, i.e. P transfer via cover crop residues, organic anion exudates, root-exuded enzymes, and microbial interactions, may happen simultaneously and warrant further investigation.
- Cover crop biomass determines in many cases the magnitude of its effect, because, in addition to the transfer of P in plant residues to the next crop, it affects the potential for rhizosphere modifications and microbial interactions. Appropriate management of the cover crop is required, acknowledging its importance to the overall return of the rotation. To determine suitable cover crop mixtures and management, more interdisciplinary projects are required.

- In order to advance our understanding of cover-crop-related effects, we suggest for future research that comparability among studies through the inclusion of appropriate controls and additional data be improved, i.e. biomass and P content of cover crops and main crops, as well as soil biological parameters and soil P pools.

Bearing these suggestions in mind, scientists will be able to join farmers in moving towards cropping systems that also improve the soil, through relevant research on the benefits of cover cropping.

5.7 Acknowledgements

Hans-Peter Piepho and Fillippo Cappezzone from the Department of Biostatistics of the University of Hohenheim provided advice on the model structure. Sven Marhan and Christian Poll gave valuable comments on an earlier version of the manuscript. Aran Berihuete Hallama kindly produced the drawings. Moritz Hallama received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 677407 (SoilCare project).

5.8 Supplementary Material

Supplementary Material S5.1 *Overview of the studies used for the meta-analysis. Available at the public repository Open Science Framework (https://osf.io/nr7km/)*

Supplementary Material S5.2 *Database of the meta-analysis. Available at the public repository Open Science Framework (https://osf.io/nr7km/)*

Supplementary Material S5.3 *Structure of the fitted models and F-Tests of the ANOVAs of the fixed effects. Available at the public repository Open Science Framework (https://osf.io/nr7km/)*

Supplementary Material S5.4 *Sample R-code of the statistical analysis. Available at the public repository Open Science Framework (https://osf.io/nr7km/*

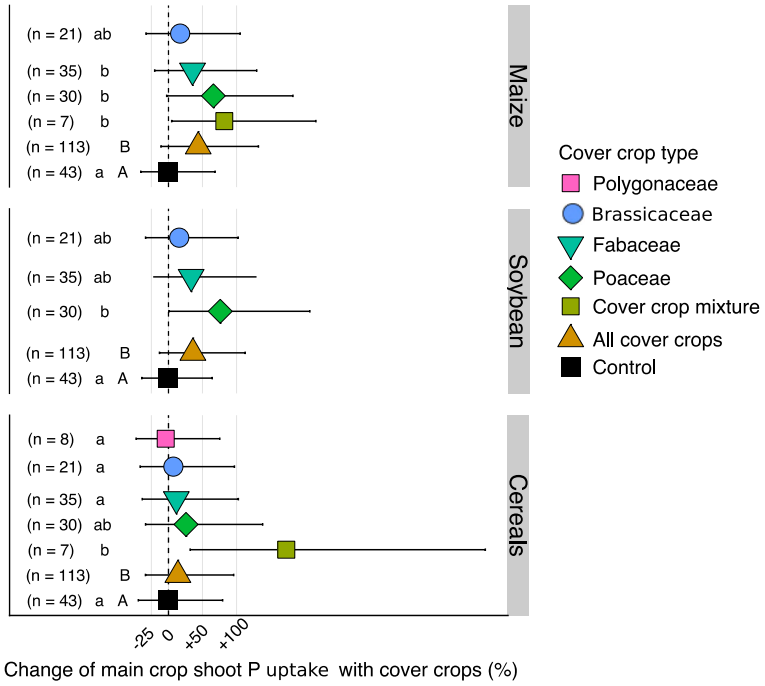


Figure S5.5 *Change in main crop phosphorus (P) content after cover crops belonging to different families. The points represent the modeled median (+/- 95 % CI) relative to the respective controls, averaged over all main crops. On the left are displayed the number of observations. The lower-case letters indicate, for a single main crop type with a Tukey post-hoc test (p<0.05), significant differences among cover crop types (including the control), and the upper-case letters differences between cover cropping in general and the controls. The corresponding models are presented in Supplementary Material S5.3.4*

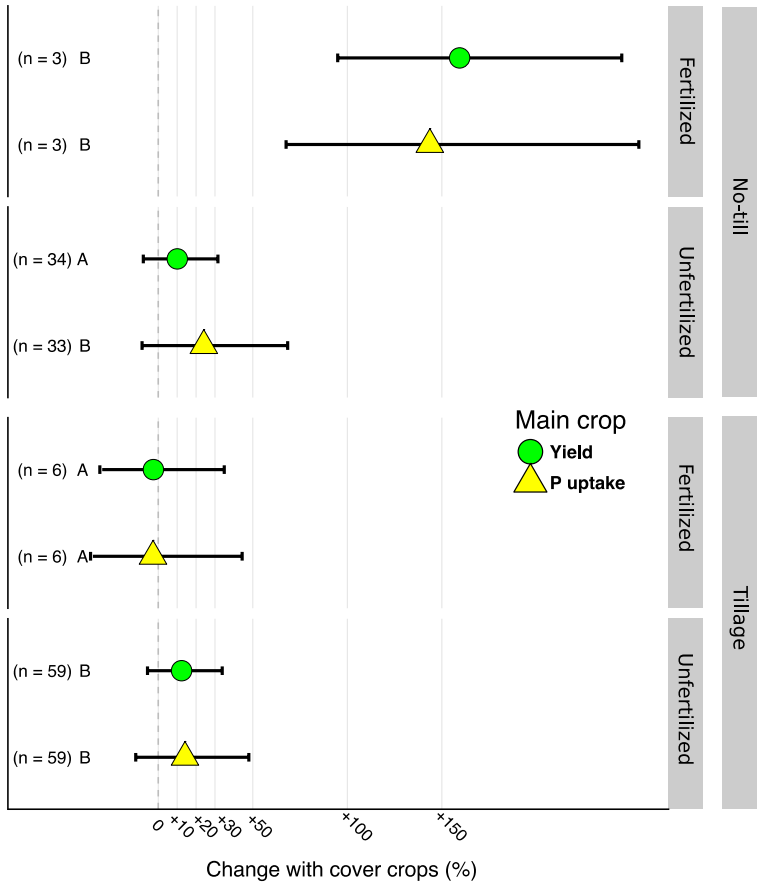


Figure S5.6 *Main crop yield and phosphorus (P) uptake as affected by cover cropping under different management regimes: noninversion tillage plus fertilization; noninversion tillage without fertilization; conventional tillage plus fertilization and conventional tillage without fertilization. Care has to be taken in the interpretation of the results of the no-till plus fertilization treatments due to the low number of observations. The points represent the percentage change of the modeled median (+/- 95 % CI) of the cover crop treatments relative to the controls without cover crops. On the left are displayed the number of observations. The letters indicate significant differences among groups with a Tukey post-hoc test (p<0.05). The corresponding models are presented in Supplementary Material S5.3.8.*

6 Study #2: The role of microbes in the increase of organic phosphorus availability in the rhizosheath of cover crops

Submitted to Plant and Soil

Submitted: 24. September 2021; currently under revision

Moritz Hallama[1], Carola Pekrun[2], Paula Mayer-Gruner[1], Marie Uksa[3], Yulduz Abdullaeva[4], Stefan Pilz[5], Michael Schloter[6], Hans Lambers[7], Ellen Kandeler[1]

[1] Soil Biology Department, Institute of Soil Science and Land Evaluation, University of Hohenheim, Emil-Wolff-Str. 27, 70599 Stuttgart, Germany

Corresponding author: hallama@uni-hohenheim.de

[2] Institute of Applied Agricultural Research, Agronomy and Quality Management, Nuertingen-Geislingen University, Neckarsteige 6-10, 72622 Nuertingen, Germany

[3] Department of Environmental Microbiology, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany

[4] Institute of Applied Microbiology, Justus-Liebig-University, Giessen, Germany

[5] Institute of Soil Science and Land Evaluation, Soil Biogeophysics Department, University of Hohenheim, Emil-Wolff-Str. 27, 70599 Stuttgart; Stuttgart, Germany

[6] Institute for Comparative Microbiome Analysis, Helmholtz Center for Environmental Health, GmbH, 85758 Oberschleissheim, Germany

[7] School of Biological Sciences and Institute of Agriculture, University of Western Australia, 35 Stirling Highway, Crawley (Perth), WA 6009, Australia

6.1 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 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.

6.2 Introduction

Conventional agricultural management requires a re-thinking 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. 2020a). 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:

1. The selected cover crops increase labile P_{org} derived from microbial necromass or rhizodeposition in their rhizosheath;
2. Cover crop species differ in their plant-microbe interactions, leading to a distinct microbial community and activity in their rhizosheath;
3. 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;
4. Soybean as a subsequent main crop benefits from the increase in labile P_{org} and microbial activity by the cover crops.

6.3 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 using direct seeding for around 12 years 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.

Experimental design

The crop rotation for the experiment was spring barley (*Hordeum vulgare* var. *Avalon*)– cover 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. 6.1.

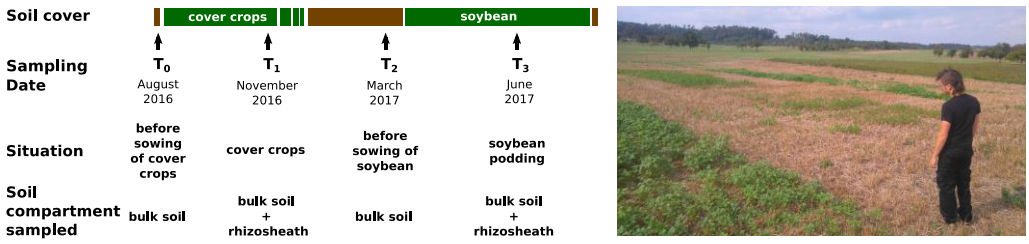


Figure 6.1 Soil cover, sampling scheme and a view of the field experiment of Study #2.

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 x 25 x 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 H_2O), 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 (P_{mic}) 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-acetyl-

hexosaminidase (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-minute 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 fatty acids and neutral lipid 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 10-fold 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%.

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 6.2. 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. 2019a), *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).

6.4 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 6.2a-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.

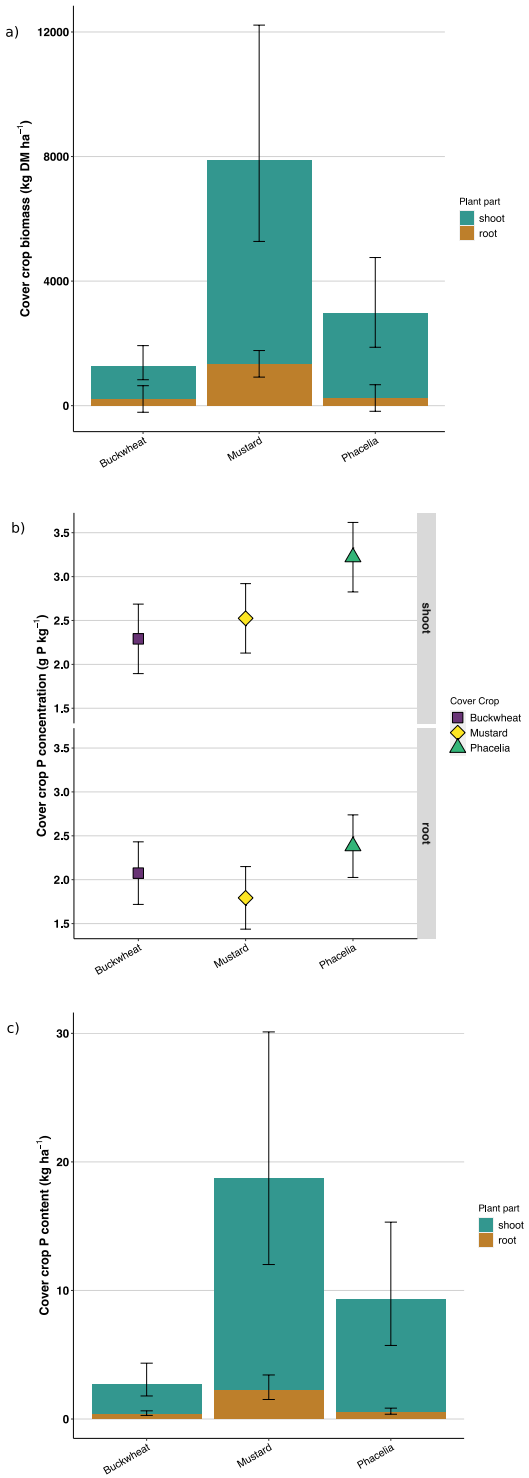


Figure 6.2 *Cover crop shoot and root parameters: a) plant biomass; b) phosphorus (P) concentration and c) plant P content. Displayed are the estimated marginal means of the four field replicates; error bars indicate the modelled 95% CI. The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

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. S6.4).

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. 6.3a). 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. 6.3b).

The enzyme-labile P_{org} pools tended to be higher in the rhizosheath of cover crops compared with the fallow control (Fig. 6.3b). 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.

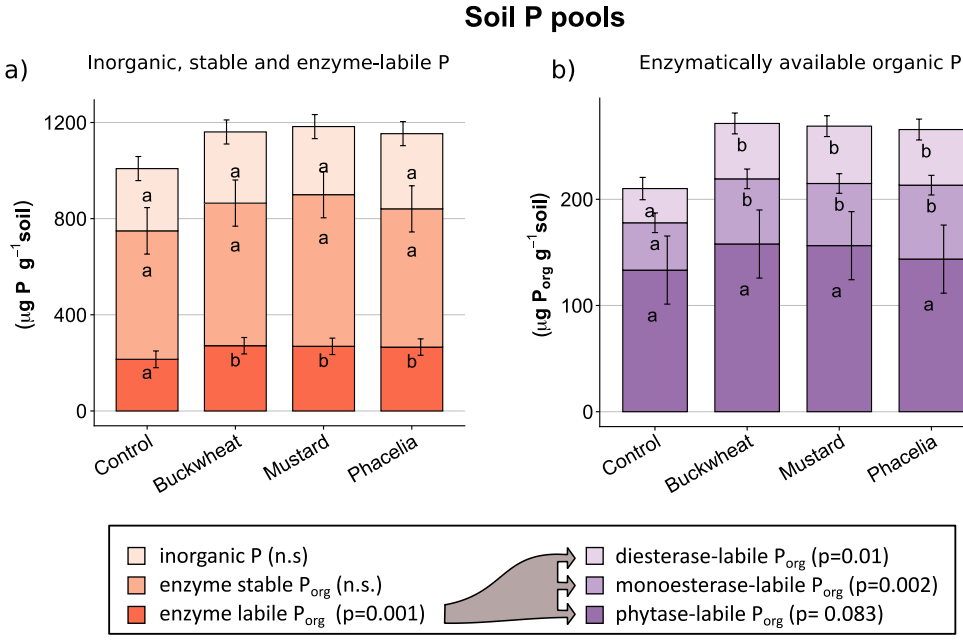


Figure 6.3 Soil phosphorus (P) pools in rhizosheath soil of buckwheat, mustard and phacelia as cover crops and bulk soil of the fallow control: a) inorganic, enzyme-stable organic P (P_{org}) and enzyme-labile P_{org} in NaOH-EDTA soil extracts; b) detailed characterisation of the enzyme-available P_{org} 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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3

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. S6.5, Supplementary Table S6.6). 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.

The recovery of an added phosphate spike increased in the rhizosheath of cover crops and decreased in the rhizosheath of soybean (Fig. S6.7, Supplementary Table S6.6) 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. 6.4, Supplementary

Table S6.6). We also found large increases of microbial P in the rhizosheath compared with that in bulk soil in June under soybean.

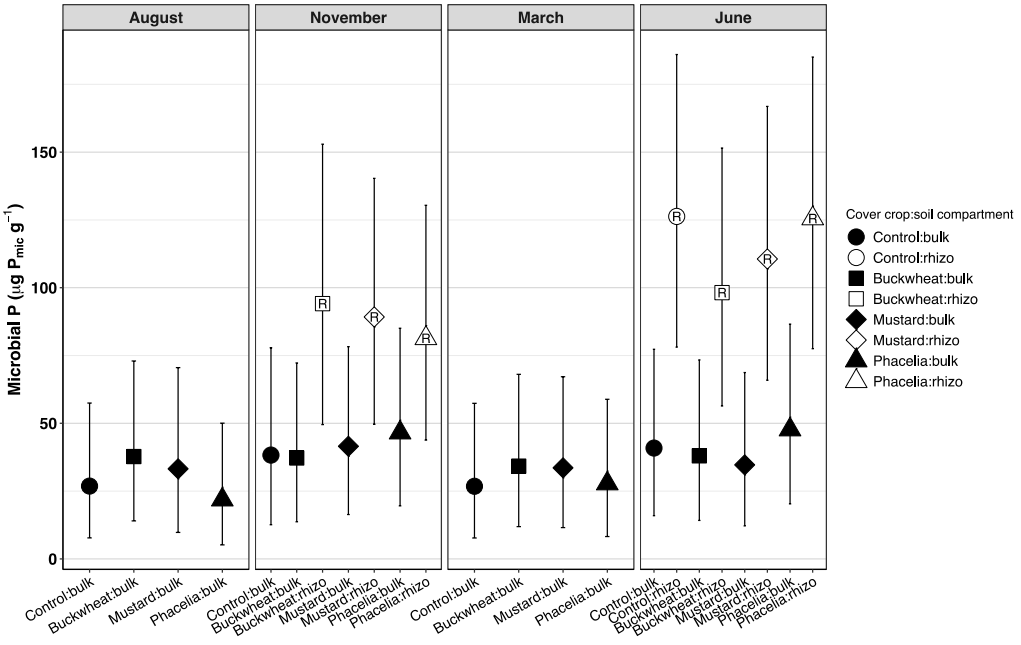


Figure 6.4 Microbial biomass phosphorus (P) in µg P g^{-1} bulk (black) and rhizosheath (white) soil of the cover crop treatments over the course of the experiment. Displayed are the estimated marginal means of the four field replicates; error bars indicate the modelled 95% CI. The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3

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 6.6.5a+b, Supplementary Table S6.6) 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. 6.5c). 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.

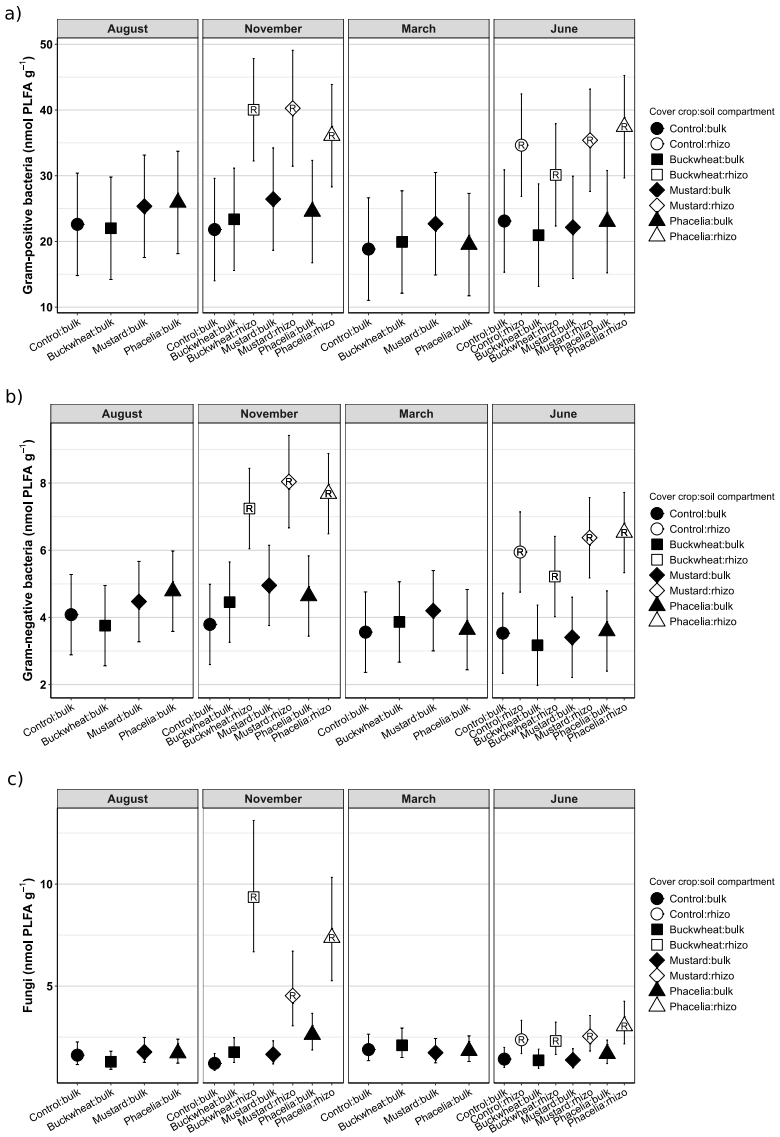


Figure 6.5 Abundance of microbial groups: a) Gram-positive bacteria [PLFAs i15:0, a15:0, i16:0, and i17:0], b) Gram-negative bacteria [PLFAs cy17:0 and cy19:0], and c) saprotrophic fungi [PLFA 18:2ω6,9] in nmol of fatty acids per gram bulk (black) and rhizosheath (white) soil of the cover crop treatments 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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3

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 6.S8 and S9).

Microbial activity

Potential enzyme activities were higher in the rhizosheath of cover crops than in the bulk soil (Figs 6.6a-d, Supplementary Table S6.10). The cover crops showed different activities of N-acetyl-hexosaminidase 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 6.S11b+c) was lower in the rhizosheath than in the bulk soil, while the specific acid phosphomonoesterase activity was not influenced by soil compartment (Fig. S6.11a).

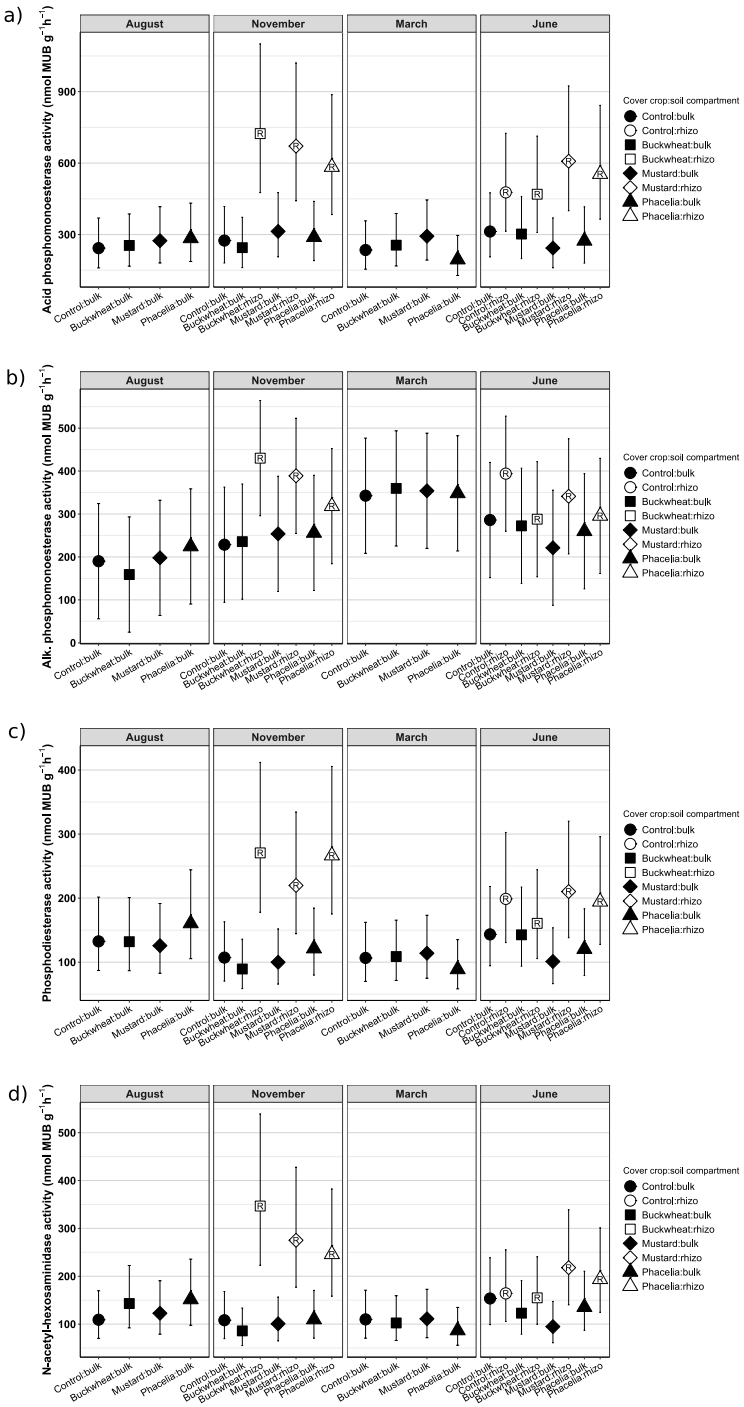


Figure 6.6 Potential activities of extracellular enzymes: a) acid phosphomonoesterase; b) alkaline phosphomonoesterase; c) phosphodiesterase and d) N-acetyl-hexosaminidase in nmol (MUB= fluorescent methylumbelliferone) product per gram dry soil per hour in bulk (black) and rhizosheath (white) soil of 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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3

Correlation of microbial community structure and function

The abundance of *phoD* copies per g soil was positively correlated with alkaline phosphomonoesterase activity (Fig. 6.7a, R^2=0.39, p<0.001) and the abundance of bacterial PLFAs (Fig. 6.7b, R^2=0.42, p<0.001). Alkaline phosphomonoesterase activity was positively correlated with bacterial PLFAs (Fig. 6.7c, 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. 6.7d, $p_{cover\ crop}$=0.046). The N-acetyl-hexosaminidase activity was strongly correlated with fungal abundance in the rhizosheath, but not at all in the bulk soil (Fig. S6.12, R^2= 0.6 and 0.0003, p<0.001 and 0.9, respectively).

The potential activity of acid phosphomonoesterase and phosphodiesterase activity 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. 6.8b).

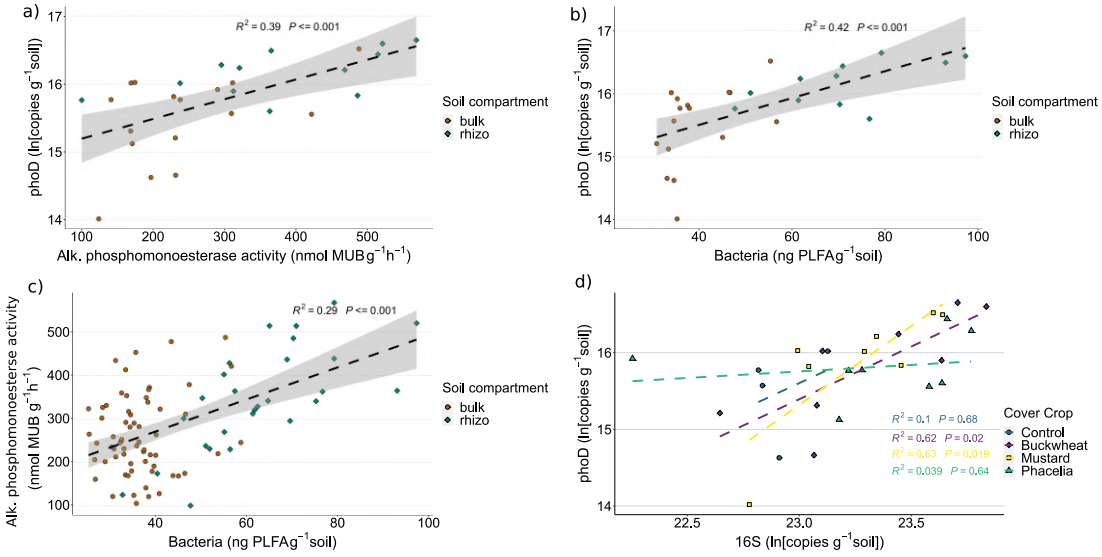


Figure 6.7 Relation of the measured potential alkaline phosphomonoesterase activity with phoD, coding for alkaline phosphomonoesterase (a); bacterial PLFA with phoD (b); bacterial PLFAs with alkaline phosphomonoesterase activity (c); and abundance of the bacterial gene phoD with the abundance of bacterial 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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3

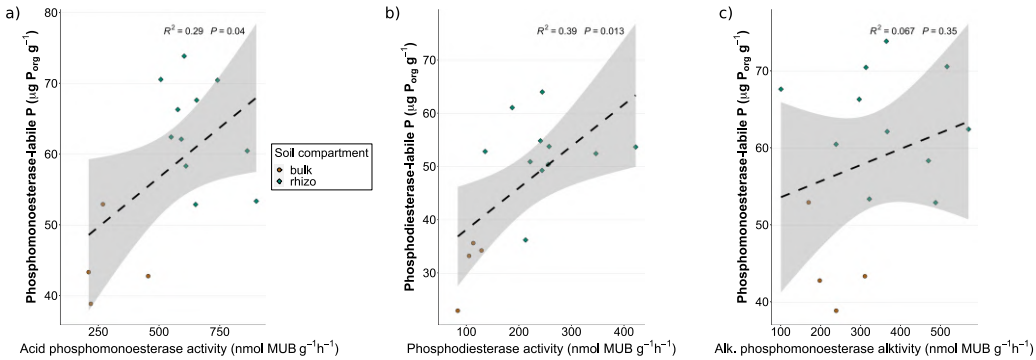


Fig 6.8 Relation of measured potential enzyme activities of a) acid phosphomonoesterase, b) alkaline phosphomonoesterase and c) phosphodiesterase the corresponding enzyme-labile organic phosphorus (P) (amount P_{org} mineralised by the addition of phosphomonoesterase or phosphodiesterase in the enzyme addition assay). The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3

6.5 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 biomass (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 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. 2012a). The amount of enzyme-labile P_{org} as quantified in the enzyme addition assay was high in comparison with that in other studies (Jarosch et al. 2015), although to our knowledge this is the first time this method was applied to rhizosheath soil. The large enzyme-labile P_{org} pool indicates a high potential for soil microorganisms to have access to this fraction. Despite these high absolute pool sizes, 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.

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 (2013a) 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 microbial activity 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 6.7b and d), an increased expression of the gene (i.e. more alkaline phosphomonoesterase activity per *phoD*) (Fig. 6.7a), or an increased specific enzyme activity per unit of microbial abundance (Figs 6.7c and S6.11b), 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. 6.7d) 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. S6.11b). 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. S6.7), associated with an increased concentration of plant-available phosphate (P_{resin}, Fig. S6.5). 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 2013a).

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).

6.6 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. 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.

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.

6.7 Acknowledgements

We thank Doreen Berner, Sabine Rudolph, and Heike Haslwimmer for assistance in the laboratory, as well as Hans-Peter Piepho for statistical support. We want to especially mention the two farmers, Alfons Bunk and Tobias Heumesser, for their support with

fieldwork. Additional gratitude goes to Julie Christensen for helpful comments during the pre-submission review. Moritz Hallama received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 677407 (SoilCare project). The field experiment formed part of the project *Konservierender Ackerbau (Conservation Agriculture),* funded by the Ministry of Agriculture and Consumer Protection of Baden-Wuerttemberg, Germany.

6.8 Supplementary Material

Supplementary Material S6.1 Excel file with the full dataset. Available at the public repository *Open Science Framework* (https://osf.io/yh5ra/)

Supplementary Material S6.2 Details of the fitted models and full ANOVA tables. Available at the public repository *Open Science Framework* (https://osf.io/yh5ra/)

Supplementary Material S6.3 R-code of the statistical analyses. Available at the public repository *Open Science Framework* (https://osf.io/yh5ra/)

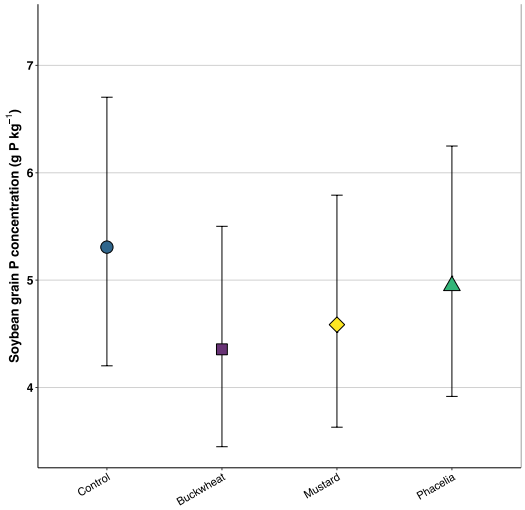


Figure S6.4 *Phosphorus (P) concentration of harvested soybean grains in mg P kg^{-1}. Displayed are the estimated marginal means of the four field replicates; error bars show the modelled 95% CI. The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

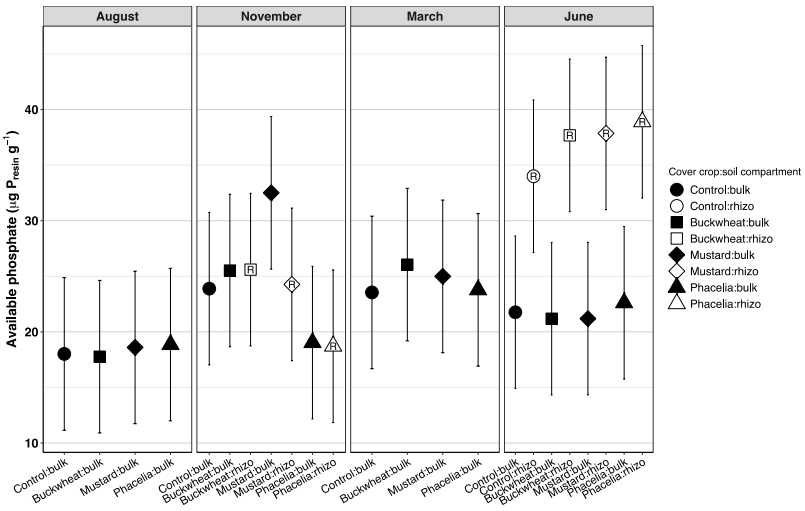


Figure S6.5 *The concentration of resin-extractable available phosphorus (P_{resin}) [µg P g^{-1} soil] in bulk (black) and rhizosheath (white) soil of the cover crop treatments over the course of the experiment. Displayed are the estimated marginal means of the four field replicates; error bars indicate the modelled 95% CI. The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Supplementary Material S6.6 *Overview of the p-values for main effects and interactions of the fitted models of P_{resin} (resin-extractable inorganic phosphorus (P)), P_{mic} (microbial biomass P), P_{rec} (recovery of added P spike, indicates P-sorption capacity) and abundances of fatty acid biomarkers of different microbial groups. The factor levels were: soil compartment (rhizosheath vs bulk soil), cover crop treatment (buckwheat, mustard, phacelia and fallow control), date (August and November 2016, March and June 2017). 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 R-code in Supplementary Material 3*

Main effects and interactions	P_{resin}	P_{mic}	P_{rec}	Gram-positive bacteria	Gram-negative bacteria	Fungi	AMF	PLFA 16:1 ω5
Soil compartment (Rhizo)	0.008	0.007	n.s.	0.004	0.0004	<0.0001	0.006	0.006
Cover Crop (CC)	0.15	n.s.	n.s.	n.s	0.01	0.008	n.s	n.s
Date	<0.0001	0.049	n.s.	0.02	<0.0001	0.002	<0.0001	0.07
CC:Rhizo	n.s.	n.s.	n.s.	n.s	n.s	n.s	n.s	n.s
Rhizo:Date	<0.0001	n.s.	0.001	n.s	n.s	<0.0001	0.051	n.s
CC:Date	0.11	n.s.	n.s.	n.s	n.s	0.003	n.s	n.s
CC:Rhizo:Date	n.s.	n.s.	n.s.	n.s	n.s	0.038	n.s	n.s

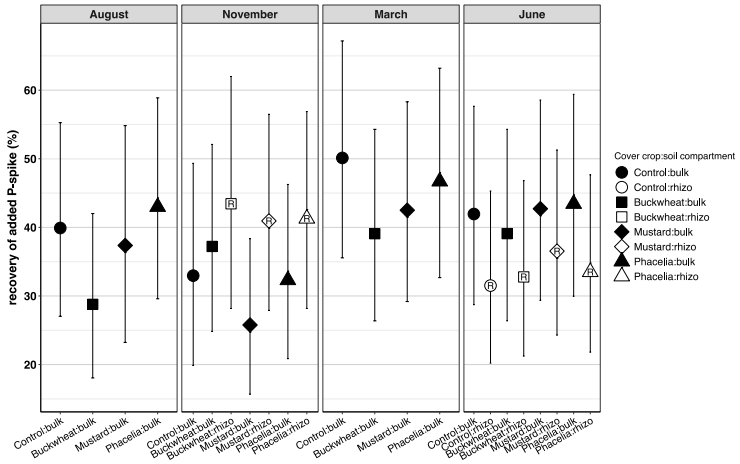


***Figure S6.7**: Recovery of added phosphorus (P) spike in %, the inverse of soil P-sorption capacity in bulk (black) and rhizosheath (white) soil of the cover crop treatments over the course of the experiment. Displayed are the estimated marginal means of the four field replicates; error bars indicate the modelled 95% CI. The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

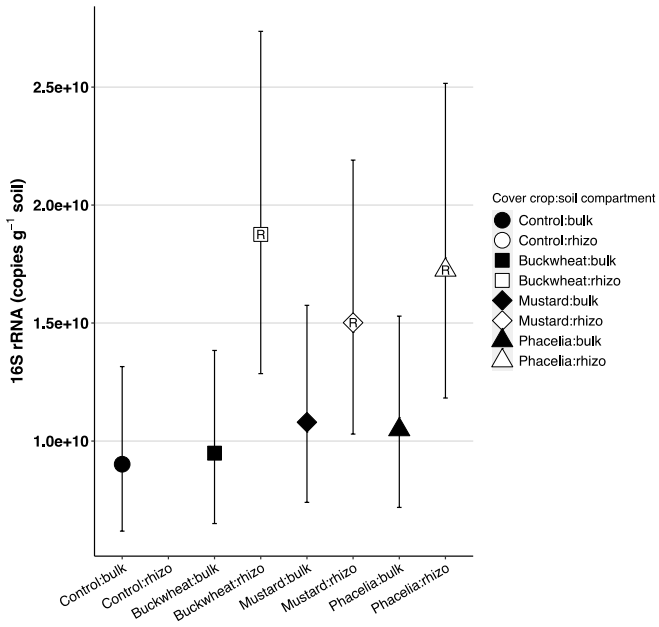


***Fig S6.8** Bacterial 16S rRNA per gram soil soil in bulk (black) and rhizosheath (white) soil of the cover crop treatments in November 2016. Displayed are the estimated marginal means of the four field replicates; error bars indicate the modelled 95% CI The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

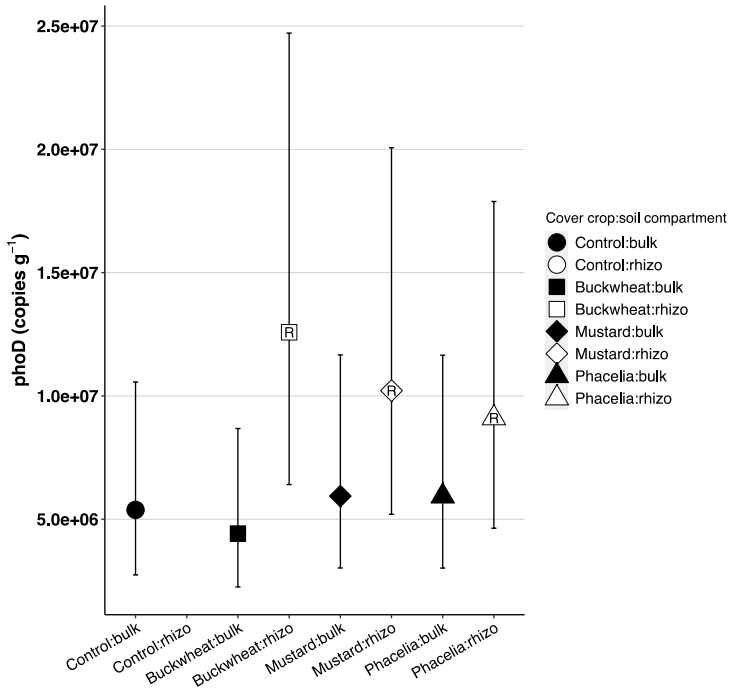


Figure S6.9 *Bacterial* phoD *gene abundance in copy numbers per gram dry soil in bulk (black) and rhizosheath (white) soil of the cover crop treatments in November 2016. Displayed are the estimated marginal means of the four field replicates; error bars indicate the modelled 95% CI. The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

Supplementary Material S6.10 *Overview of the p-values for main effects and interactions of the fitted models of different enzyme activities presented in Fig. 6.6. The factor levels were: soil compartment (rhizosheath vs bulk soil), cover crop treatment (buckwheat, mustard, phacelia and bare fallow control), date (August and November 2016, March and June 2017).* *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 R-code in Supplementary Material 3*

Main effects and interactions	Acid phosphomonoesterase	Alkaline phosphomonoesterase	Phosphodiesterase	N-acetyl-hexos-aminidase
Soil compartment (Rhizo)	0.0006	0.072	<0.0001	0.005
Cover Crop (CC)	n.s.	n.s.	n.s.	n.s.
Date	n.s.	<0.0001	0.07	n.s.
CC:Rhizo	n.s.	n.s.	n.s.	0.043
Rhizo:Date	n.s.	0.078	0.0036	<0.0001
CC:Date	n.s.	n.s.	n.s.	0.10
CC:Rhizo:Date	0.093	n.s.	0.079	0.013

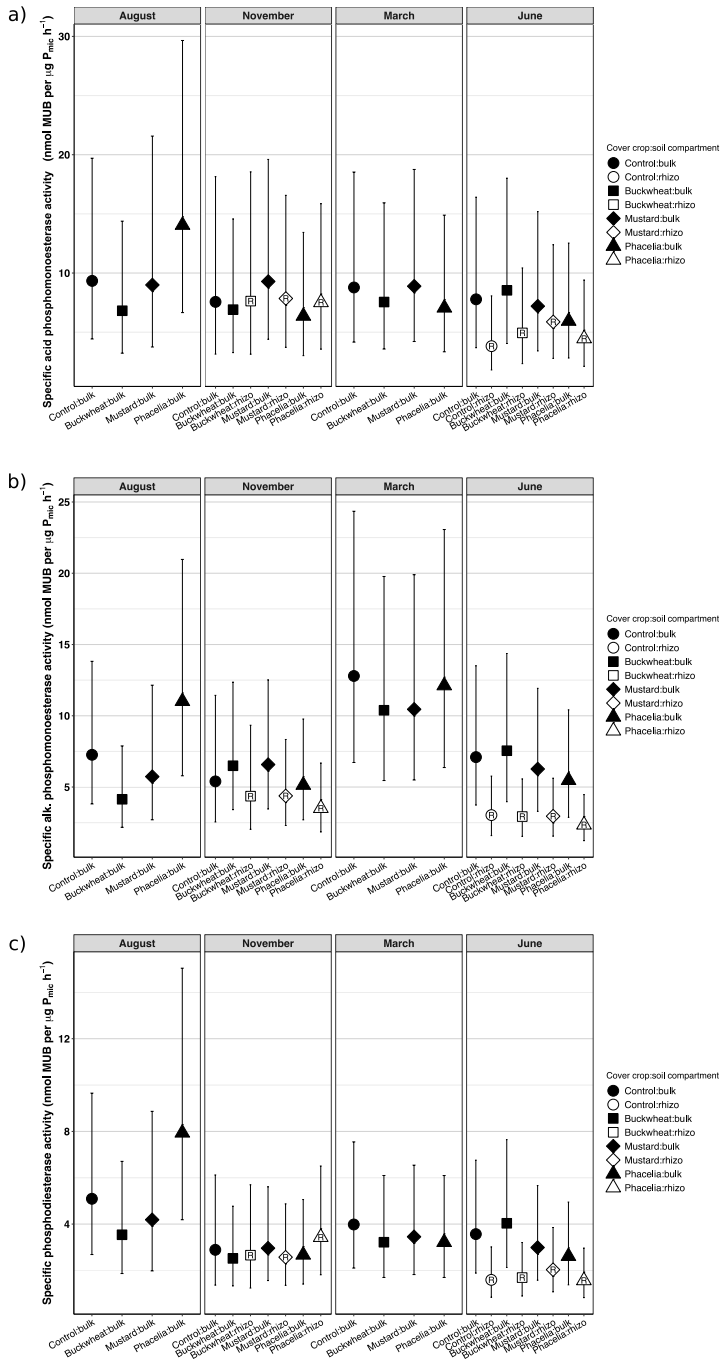


Figure S6.11 *Ppotential activities of extracellular phosphatase enzymes per microbial biomass phosphorus (P): a) acid phosphomonoesterase; b) alkaline phosphomonoesterase and c) phosphodiesterase in nmol of (fluorescent) substrate µg P_{mic} per hour in bulk (black) and rhizosheath (white) soil of the 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 S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

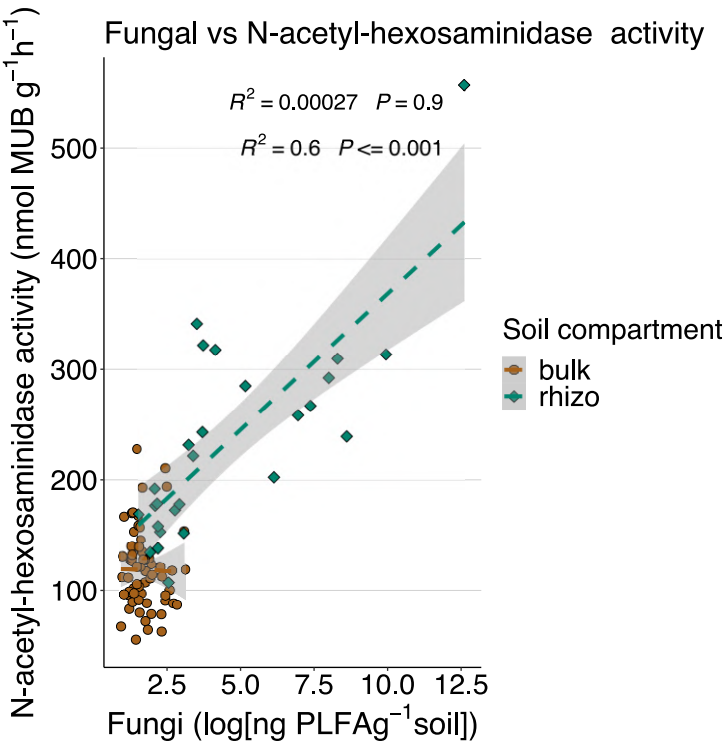


Figure S6.12 *Relation of the abundance of fungal PLFA 18:2ω6,9 with measured potential N-acetyl-hexosaminidase (NAGase) activity. The underlying data is provided in Supplementary Material S6.1, the structure of the fitted models and the F-tests in Supplementary Material S6.2 and the complete R code in Supplementary Material S6.3*

7 Study #3: Interactions between cover crops and soil microorganisms increase phosphorus availability in conservation agriculture

Hallama, M., Pekrun, C., Pilz, S. *et al.* Interactions between cover crops and soil microorganisms increase phosphorus availability in conservation agriculture. *Plant Soil* **463,** 307–328 (2021). https://doi.org/10.1007/s11104-021-04897-x

Received: 24 August 2020; Accepted: 22 February 2021

Published: 22 March 2021; Issue Date: June 2021

Moritz Hallama[1], Carola Pekrun[2], Stefan Pilz[2], Klaus Jarosch[3], Magdalena Frąc[4], Marie Uksa[1], Sven Marhan[1], Ellen Kandeler[1]

[1] Soil Biology Department, Institute of Soil Science and Land Evaluation, University of Hohenheim, Emil-Wolff-Str. 27, 70599 Stuttgart;

Corresponding author: hallama@uni-hohenheim.de

[2] Agronomy Section, Institute of Applied Agriculture, Nuertingen-Geislingen University, Neckarsteige 6-10, 72622 Nürtingen, Germany

[3] Institute of Geography, University of Bern, Hallerstrasse 12, 3012 Bern, Switzerland

[4] Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland

7.1 Abstract

Aims: An essential task of agricultural systems is to improve internal phosphorus (P) recycling. Cover crops and tillage reduction can increase sustainability, but it is not known whether stimulation of the soil microbial community can increase the availability of soil organic P pools.

Methods: In a field experiment in southwest Germany, the effects of a winter cover crop mixture (vs. bare fallow) and no-till (vs. non-inversion tillage) on microbial P-cycling were assessed with soybean as the main crop. Microbial biomass, phospholipid fatty acids (PLFAs), P cycling enzymes, and carbon-substrate use capacity were linked for the first time with the lability of organic P pools measured by enzyme addition assays (using phosphodiesterase, non-phytase-phosphomonoesterase and fungal phytase).

Results: Microbial phosphorus, phosphatase, and fatty acids increased under cover crops, indicating an enhanced potential for organic P cycling. Enzyme-stable organic P shifted towards enzyme-labile organic P pools. Effects of no-till were weaker, and a synergy with cover crops was not evident.

Conclusions: In this experiment, cover crops were able to increase the microbially mediated internal P cycling in a non-P-limited, temperate agroecosystems.

7.2 Introduction

Crop production depends on a sufficient supply of major nutrients such as phosphorus (P). Improving the internal recycling of P in agroecosystems is needed and this is especially urgent in agroecosystems with a long history of P fertilisation, in order to reduce dependence on diminishing mineable P resources (Carpenter and Bennett 2011; Schröder et al. 2011), and to reduce detrimental effects that losses of excess P to other ecosystems can have (Ceulemans et al. 2014; Sharpley 2016). In soil, P is present either in inorganic (P_i) or organic (P_{org}) forms. Typically in agricultural temperate soils, only about 5% of total soil P is dissolved in soil solution and thereby available for plant uptake in the form of ortho-phosphate (Stutter et al. 2015). Consequently, the soil solution has to be continuously replenished with orthophosphate, either by desorption processes from the soil mineral phase or by mineralization of organic P.

In industrialised countries, past organic and mineral fertilizer applications to agricultural soils have led to an accumulation of "residual P" or "legacy P", which is composed of inorganic and organic P of limited availability (Stutter et al. 2015; Lemming et al. 2019). The residual P can be considered as a potential resource and its improved use could reduce dependence of modern agriculture on fertilizer inputs (Menezes-Blackburn et al. 2018). In recent years, management of soil organic P dynamics has received particular attention (George et al. 2018), since soil organic P can comprise between approximately 30 and 80% of total soil P (Harrison 1987). A large proportion of organic P in soil is bound as monoesters in supramolecular structures (McLaren et al. 2015), phytates, non-phytate monoesters, and diesters (Turner et al. 2007). Plant-available orthophosphate can be released from P_{org} in a process catalysed by different phosphatase enzymes produced by soil biota (Harrison 1987). Phosphomonoesters (e.g., inositol phosphates/phytates, sugar phosphates, and mononucleotides) are dephosphorylated by phosphomonoesterases, whereas for diesters (e.g., nucleic acids and phospholipids) an initial hydrolysation by a phosphodiesterase is required. Phytases represent a specialized form of phosphomonoesterases additionally capable of initiating the cleavage of higher-order inositols (Konietzny and Greiner 2002). While some plants are capable of producing phosphomonoesterases, they do not release significant amounts of phosphodiesterases or phytases (Turner and Haygarth 2005), making soil microorganisms the main source of these enzymes and therefore the key drivers of mineralisation of organic P compounds (Bünemann et al. 2007; Richardson and Simpson 2011). The mobilisation of P_i and P_{org} is affected by the production and degradation of P-mobilising compounds by microbes (Jones and Oburger 2011). Additionally, soil microbes affect the P nutrition of plants via antagonistic effects on plant pathogens (Finckh et al. 2019), as well as production of phytohormones that modify both root growth and architecture (Hayat et al. 2010). Among these microbes, arbuscular mycorrhizal fungi (AMF) are the most studied, and their abundance can be directly related to improved P nutrition for plants, especially in P-limited agroecosystems (Jansa et al. 2011; Cozzolino et al. 2013).

Cropping systems that enhance soil microorganisms' capacity to improve the efficient management of nutrients and the use of residual P by mobilising P_i and mobilising and mineralising P_{org} pools can be an option for a wide range of agroecosystems, from nutrient

limited soils in the tropics to heavily fertilized temperate agroecosystems (Oberson et al. 2006; Wendling et al. 2016). Conservation agriculture, consisting of cover cropping in combination with tillage reduction, is such an option, providing multiple benefits to both soil fertility and to the environment (Hobbs et al. 2008; Büchi et al. 2018), as well as closing gaps in P cycling. Recently, Hallama et al. (2019) described three pathways of cover crop-derived P benefit for the main crop in a meta-analysis. First, nutrients are taken up from the soil and stored in the cover crop plant tissues, released after their mineralisation in spring. Second, cover crops interact with the soil microbial community, shaping its abundance, structure and functions, potentially increasing the P supply to the main crop (Deubel and Merbach 2005; Oberson et al. 2006). Finally, some cover crops, especially lupines, can modify the soil chemistry in their rhizosphere, mobilizing P sources that are otherwise limited (Lambers et al. 2013). Previous studies of P-cycling in agroecosystems focused either on chemical or microbiological soil properties, whereas the complex interactions between P-cycling microorganisms and the lability of different P fractions in soil have been less well studied (Frossard et al. 2000; George et al. 2018).

In order to test the validity of the pathways of cover crop-derived P-benefit mentioned in Hallama et al. (2019), the aim of the current study was to clarify whether conservation agriculture, with its component cover crops and no-till, stimulates microbial abundance and function and changes the lability of the P_{org} fractions. Under conservation agriculture, an enhanced microbial community may lead to increased storage of P in living and dead biomass, resulting in a shift from P_i and P_{org} fractions with limited availability to more labile P_{org} fractions. Thus, we hypothesize that under conservation agriculture (cover crop/no-till): (1) soil P shifts towards more available pools; (2) a stimulated microbial community with enhanced functions is associated with changed P pools, and; (3) cover crops and no-till may have synergistic effects on soil microbial biomass, microbial community structure, and P-cycling capacity.

To evaluate P dynamics under field conditions and to gain a more detailed understanding of the link between the function of P cycling microorganisms and the potential lability of organic P compounds, an enzyme addition assay (EAA) was used. This biochemical method consists in the addition of enzymes targeting specific P classes and quantifies the

hydrolysabilty of specific P_{org} classes by substrate specific enzymes (Bünemann 2008). The relationship between enzyme activities and the soil microbial community was investigated by quantifying the total microbial P pool as well as the different microbial groups of soil organisms by analysis of neutral and phospholipid fatty acids.

7.3 Materials and Methods

Site description

The field experiment was conducted at the Tachenhausen Experimental Farm near Stuttgart, Germany (48.649800 N, 9.387500 E, 330 m a.s.l.) and was established in autumn 2012. The soil is a Stagnic Cambisol (IUSS Working Group WRB 2015) with a very fine sandy loam texture. The field has an average $pH_{(H2O)}$ of 6.5, a soil organic carbon content of 14 g kg^{-1} soil and a rather high P_{CAL}, averaging 108 mg kg^{-1} soil. The climate is temperate with a mean annual temperature of 8.8º C and 809.3 mm precipitation (monitoring station Wetterstation Tachenhausen HfWU, 200 m from the site, 1961-1990). The field has a history of conventional agriculture, with a crop rotation consisting mainly of cereals and winter oilseed rape. The crop rotation for the experiment was winter wheat – cover crop mixture – soybean. An overview of climate and management is presented in Fig. 7.1; a detailed list with field observations and the agronomic management can be found in Supplementary Material S7.7.1.

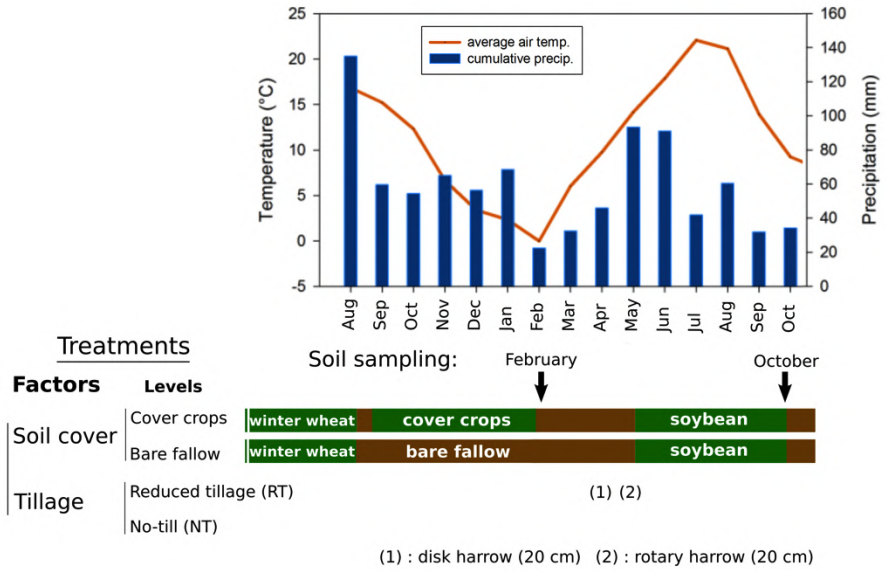


Figure 7.1 *Climate and management of the field experiment. Top: climate chart (left y-axis: monthly average air temperature [°C], right y-axis: cumulative monthly precipitation [mm]). Bottom: sampling (February and October 2015), soil cover and management (RT: reduced tillage). Further management details are listed in Supplementary Material S7.1*

In the field trial, the effects of tillage and soil coverage on soil properties were compared in a full factorial design. Tillage consisted of either reduced (non-inversion) tillage (RT) or no-till/direct seeding (NT), while soil coverage included either a bare fallow or a cover crop mixture. The field trial was replicated with three complete blocks. To simplify handling of field operations, the experiment was set up in a split-plot design, with the levels of tillage randomly allocated to two main plots within each of the three blocks and the levels of cover crops randomized as two subplots (strips of 6 m by 100 m) within each main plot, resulting in a total of 12 plots. Conservation agriculture management consists of the simultaneous use of direct seeding and cover crops. Although tillage effects probably would have been greater with the extreme comparison of deep inversion tillage and no-till, the more modern non-inversion tillage approach was used as a control, as it is becoming standard in the region. In the cover crop treatments, a commercially available mixture (Terra Life Beta Maxx® 2014 provided by Deutsche Saatveredelung AG, Germany), containing *Trifolium alexandrinum, Pisum arvense, Vicia sativa, Lupinus angustifolius, Guizotia abyssinica and Phacelia tanacetifolia* was direct seeded at a rate of 45 kg ha^{-1}. This specific mixture including legumes was considered a compromise between positive effects on soil structure,

N supply, winter-killing and only a minor risk of pathogens for the main crops. At the end of the vegetation period in November 2014, the cover crop biomass of RT and NT was rather low with 1114 and 1689 kg dry mass ha^{-1}, respectively. The field emergence and biomass production of the cover crop species in the mixture can be found in Table S1. Despite repeated applications of herbicides, weed pressure was generally high. Rabbits, mice and snails constituted an additional problem for the cover crops.

Soil samples were taken in February 2015, after frost-death of the cover crops, and October, at soybean harvest, at 0-5 and 5-20 cm depths with an auger, from around eight locations inside each of the twelve plots and pooled per plot and depth. The samples were sieved at 5 mm and stored at -20º C until analysis. For the chemical determination of calcium-acetate-lactate extractable P (P_{CAL}), a standard method to estimate soil P status for crops, soil samples were dried (60º C for 72 h), milled and extracted with calcium-acetate-lactate (VDLUFA 2012).

Enzymatic availability of organic P pools

An enzyme addition assay was used to characterize different organic P forms in an alkaline soil extract, depending on their lability for enzymatic degradation (Bünemann 2008; Jarosch et al. 2015). In principle, substrate-specific enzymes are added to hydrolyse specific P_{org} compounds in soil NaOH/EDTA-extracts. The increase in molybdate-reactive P compared to an untreated control sample yields the quantity of the corresponding enzyme-labile P_{org} pool in the extract.

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

The enzyme addition assay was performed as described in Jarosch et al. (2015). In short, soil NaOH/ETDA extracts (0.25 M NaOH and 0.05 M EDTA) were incubated alone or in combination with substrate specific phosphatase enzymes. The enzymatic characterisation of the NaOH-EDTA extracts was performed under the same conditions for all enzymes in transparent 96 well microplates, adding enzymes to the NaOH-EDTA extract and MES buffer

adjusted to pH 5.2, in a final volume of 300 µl per well. The plates were incubated for 24 h at 37º C horizontally shaking at 40 rpm, transferred into another plate with malachite green and absorbance was measured as above. Two replicates of each sample were analysed in separate analysis runs.

The addition of acid phosphatase (Sigma P1146) alone quantifies non-phytate-monoester P_{org}, for which term "monoester labile P_{org}" is used (Formula 1).

$$\text{Monoesterase labile } P_{org} = P_{org} \text{ hydrolysed by acid phosphatase}$$

(Formula 1)

Phosphodiesterase-labile P_{org} was quantified by the addition of phosphodiesterase/nuclease (Sigma N8630) in combination with acid phosphatase (Formula 2), since in phosphodiesterase hydrolyses only the first of the two ester bonds in diesters, such that a phosphomonoesterase is also required to produce detectable phosphate.

$$\begin{aligned}\text{Diesterase labile } P_{org} \\ &= P_{org} \text{ hydrolysed by nuclease in combination with acid phosphatase} \\ &- \text{ monoesterase labile } P_{org}\end{aligned}$$

(Formula 2)

Two phytases, a fungal (*Peniophora lycii*, Ronozyme NP, Novozyme, Denmark) and a commercial bacterial phytase (*E. coli*, *Quantum blue*, ABVista, USA), that target overlapping phytase-labile P_{org} pools, were used in order to reflect the activities of different microbial groups (Formula 3 and 4). The pool of monoesterase labile P_{org} must be subtracted from the phosphate released by the phytases, as the added phytases also mineralise non-phytate monoesters.

$$\text{Fungal phytase labile } P_{org} = P_{rg} \text{ hydrolysed by fungal phytase } - \text{monoesterase labile } P_{org}$$

(Formula 3)

$$\text{Bacterial phytase labile } P_{org} = P_{org} \text{ hydrolysed by bacterial phytase } - \text{monoesterase labile } P_{org}$$

(Formula 4)

However, for the characterisation of the enzyme-labile and enzyme-stable P_{org} pools, only the fungal phytase was used (Formulas 5 and 6), as this specific enzyme has been employed in other studies (Annaheim et al. 2013; Jarosch et al. 2015).

$$\text{Enzyme} - \text{labile } P_{org} = \text{fungal phytase labile } P_{org} + \text{diesterase labile } P_{org} + \text{monoesterase labile } P_{org}$$

(Formula 5)

$$\text{Enzyme} - \text{stable } P_{org} = \text{Total } P_{org} - \text{Enzyme} - \text{labile } P_{org}$$

(Formula 6)

Since the calculations are based on several subtractions of P concentrations in enzyme-treated and untreated extracts, as well as background concentrations in enzyme preparations, unrealistic values were sometimes obtained. When more than three of the five analytical replicates (i.e., wells of microtiter plates) had very low or even negative values, the entire pool was set to NA (data in S2). The individual analysis run (each of the three field replicates of each treatment was analysed in two separate runs) was included as a random effect in the statistical model.

Microbial biomass P

Phosphorus bound in the microbial biomass (P_{mic}) was determined on field-moist, unfrozen soil by hexanol fumigation and simultaneous extraction with anion exchange resin membranes (Kouno et al. 1995). For this, 2.5 g dry weight base 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 spike (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 phosphate from the resins, and the P concentration was measured colorimetrically according to Murphy-Riley at 610 nm (Murphy and Riley 1962). The difference between the fumigated and the unfumigated samples (Formula 7) was used as a proxy for microbial biomass P (P_{mic}), since the high recovery rate of P_{spike} revealed a very low sorption of released phosphate

$$P_{mic} = P_{hex} - P_{resin}$$

(Formula 7)

A K_P-conversion factor to account for incomplete extraction of microbial P (Brookes et al. 1982) was not applied since it has not been determined for this specific soil (McLaughlin et al. 1986).

Microbial biomass carbon

Substrate-induced respiration (SIR) was determined to estimate microbial biomass (C_{mic}) (Anderson and Domsch 1978) using automated electrolytic microrespirometry (Respiration Measurement System, ETS, Darmstadt, Germany) (Scheu 1992). Four grams of frozen soil were weighed in plastic cups and acclimatized over 48 h at room temperature. Four µg glucose g $soil^{-1}$ were added in aqueous solution (100 µl g^{-1} soil fresh weight) and the samples were incubated for the respiration measurement at 22 °C. The initial respiration rate (average of the three lowest values within the first eight hours) was used to estimate C_{mic} using a conversion factor of 38 (Beck et al. 1997).

Potential activity of extracellular enzymes

Potential activities of acid phosphomonoesterase (EC 3.1.3.1), phosphodiesterase (EC 3.1.4.1), β-D-glucosidase (EC 3.2.1.21) and N-acetyl-glucosaminidase (EC 3.2.1.52) were determined using fluorescent 4-methylumbelliferone substrates based on Marx et al. (2001), modified by Poll et al. (2006). The substrates were obtained from Sigma–Aldrich, St.

Louis, USA, except for the phosphodiesterase substrate, which was obtained from Carbosynth, Compton, UK.

For the analysis, 1 g of soil was ultra-sonicated at 50 J s^{-1} for 120 s in 50 ml of autoclaved H_2O. Fifty µl of soil suspension, 50 µl MES buffer (0.1 M MES-buffer, pH 6.1) and 100 µl substrate were pipetted onto microplates and incubated at 30 °C. The increase in fluorescence over time (slope) was measured at 5 intervals over 180 min at 360/460 nm on a Microplate Fluorescence Reader (FLX 800, Bio-Tek Instruments, USA) and converted into nmol substrate g $soil^{-1}$ h^{-1} using a sample-specific standard curve with 4-methylumbelliferone added to the soil suspension.

Phospholipid fatty acids and neutral lipid acids

The structure of the soil microbial community was characterized by extraction and analysis of specific phospholipid fatty acids (PLFA) and neutral fatty acids (NLFA) (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 -20 º C 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, Germany). Specific biomarker fatty acids permit 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 (Gram+), and cy17:0 and cy19:0 for Gram-negative (Gram-) bacteria. The sum of these fatty acids, together with 16:1ω7 and 15:0, can be used as general bacterial biomarkers. The PLFAs 18:2ω6,9 and 18:3ω6,9,12 were used as general markers for fungi (Frostegård and Bååth 1996). The sum of the bacterial and fungal markers, together with the general microbial PLFA 16:1ω5, was used as a proxy for microbial biomass. The neutral fatty acid (NLFA) 16:1ω5 was used as a marker for arbuscular mycorrhizal fungal abundance (Olsson 1999).

Substrate use capacity expressed as metabolic potential diversity

The capacity of microbial communities to mineralise different substrates characterises functional diversity. In this study, Biolog EcoPlates (Biolog Inc., Hayward, CA) were used, in which soil suspensions are added to commercially available microplates containing a standardised set of carboxylic acids, carbohydrates, polymers, amines/amides and amino acid substrates and a colouring agent in the wells (Insam 1997; Insam and Goberna 2004). Colour development is observed when microorganisms inoculated into the wells utilize the substrates (Frąc et al. 2012).

Soil suspensions were prepared from 1 g frozen soil in 99 ml of sterile saline peptone water, shaken for 20 minutes at 20 °C and incubated at 4 °C for 30 minutes for the sedimentation of soil particles. Each well of the EcoPlates was inoculated with 120 µl of soil solution. The EcoPlates, covered by lids, were incubated at 25 °C in the dark (Gryta et al. 2014). Absorbance was measured at 590 nm at time intervals of 24 h for 9 days in a Biolog Microstation (Biolog Inc., USA). The microbial response in each well of microplates, regarded as substrate utilization, was expressed as the average well colour development (AWCD). Shannon-Weaver's diversity index (H) was calculated from the number of oxidized C substrates at the threshold of 0.25 (Gomez et al. 2006). For calculations, the average of the measurements after 72, 96 and 120 h of incubation was used.

Statistical Analysis

To account for the split-plot design (three field replicates per treatment), linear mixed models with *block* and the interactions with *depth* and *date* as fixed effects and the interaction of *mainplot* and *subplot* with *depth* and *date* as random effects (Piepho et al. 2003) were fitted using the package *lme4* v1.1-19 (Bates et al. 2015), in *R* v3.5.0 (R-Core Team 2013) and *R-Studio* v1.1.453 (RStudio 2013). Interactions with random factors were considered random according to Piepho et al. (2003). The complex structure of the models was reduced by elimination of the random effects with a standard deviation of 0, afterwards applying the step function in *R* to reduce the fixed effects but keeping the block effects. The residuals were checked using Q-Q-plots and histograms (Schützenmeister et al. 2012; Kozak and Piepho 2018). The structure of the fitted models and the F-tests are provided in

Supplementary Material S7.3, R code in Supplementary Material S7.4. The following packages were employed: *readxl* (Wickham and Bryan 2018), *openxlsx* (Schauberger and Walker 2019), *dplyr* (Wickham et al. 2019b), *stringi* (Gagolewski 2018), *tidyverse* (Wickham et al. 2019a), *pbkrtest* (Halekoh and Højsgaard 2014) and *LmerTest* (Kuznetsova et al. 2017). The figures were produced with estimated means and 95 % confidence intervals using *emmeans* (Lenth 2018) and *multcomp* (Hothorn et al. 2008) with *ggplot2* (Wickham 2009), *cowplot* (Wilke 2017) as well as *RColorBrewer* (Neuwirth 2014). The radar chart was elaborated using the package *fmsb* (Nakazawa 2018). The figures were produced with the estimated means of the full models in order to be able to show also non-significant factors, while the F-tests of the significant effects were calculated with the respective reduced models.

To simultaneously visualise and test the responses of multiple properties that characterise microbial community composition and function to the treatments, linear discriminant analysis (LDA) was used. In this dimensionality reduction technique, multiple microbial properties are loaded on the linear discriminant axes that maximise the separation between the four groups (treatments). For microbial community structure, abundances of single fatty acid biomarkers were used, while for microbial activity, enzyme activities and carbon substrate group utilisation data were used (R code in Supplementary Material S7.4).

7.4 Results

Treatment effects were more pronounced in the topsoil (0-5 cm) than in the deeper soil layers (5-20 cm). Consequently, the presentation of the results was focused on the upper 0-5 cm of the soil. Data on soil properties of 5-20 cm can be found in Supplementary Material S7.2.

Cover crops increase enzymatic availability of organic P pools

Total P in the NaOH-EDTA extracts ranged from 690-780 µg g^{-1} soil, of which around 60 % were P_i and the remaining 40 % P_{org}, (Fig. 7.2 a). Of the P_{org} pool, on average 98 µg P_{org} g^{-1}

(around 40 % of total P_{org}) were enzyme-labile, with cover crops increasing the amount of enzyme-labile P_{org} in October in comparison to bare fallow treatments (Fig. 7.2 a, Table 7.1, *Cover crop x Date* p=0.012). The largest proportion of enzyme-labile P_{org} was available for phytase. Fungal phytase-labile P_{org} was highest under cover crops and RT (Fig. 7.2 b, Table 7.1, *Cover crop x Tillage* p=0.015). A bacterial phytase hydrolysed slightly greater quantities of phytate than the fungal phytase and was highest under cover crops in October (Fig. S7.5). Phosphomonoesterase-labile P increased under cover crops in October (Fig. 7.2 b, Table 7.1, *Cover crop x Date* p=0.079). The pool of phosphodiesterase-labile P was the lowest and most variable of the pools, and showed no treatment effects (Fig. 7.2 b, Table 7.1).

The standard soil P test P_{CAL} tended to be highest in bare+NT (Fig. 7.3), whereas resin-P did not show any treatment effects (Fig. S7.6). The high values, generally above 100 mg P_{CAL} kg^{-1} soil, suggest an excess availability of P for crops.

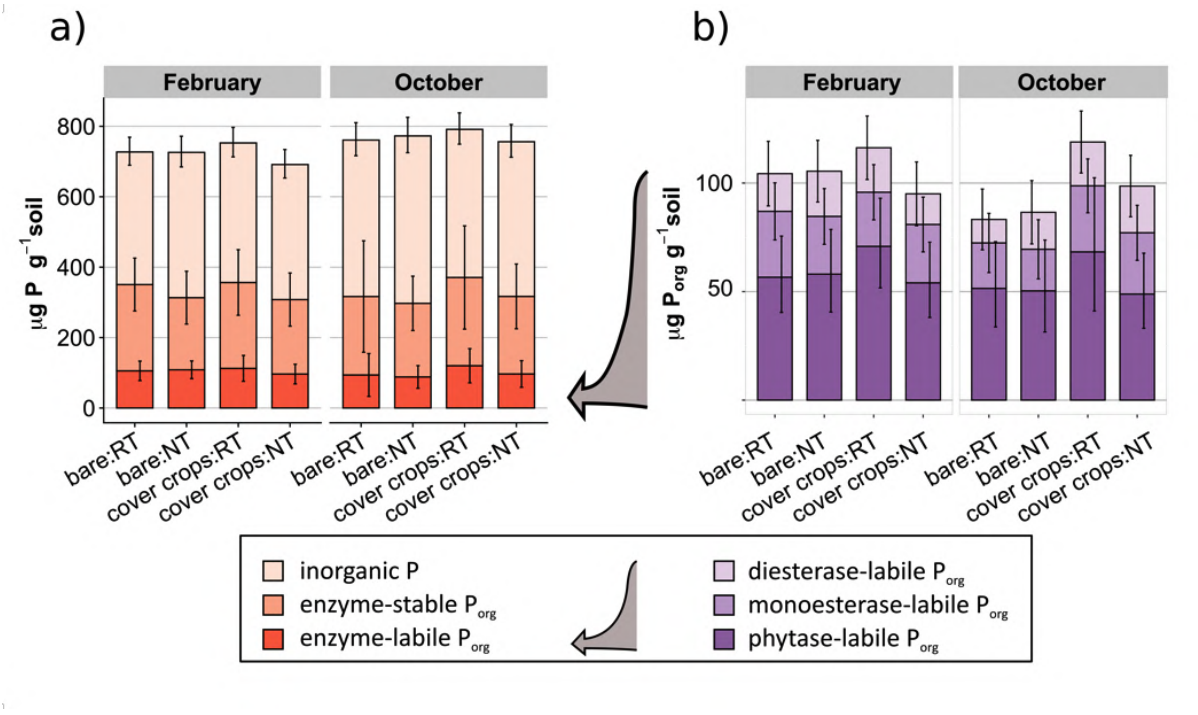


Figure 7.2 *Soil P pools at Tachenhausen field site in 0-5 cm. a) In the left figure, the top, middle and bottom bars correspond to inorganic P (P_i), enzyme-stable organic P (P_{org}) and enzyme-labile P_{org}., respectively b) The enzyme-labile P pool can be further subdivided into P_{org} hydrolysable for phosphodiesterase, non-phytase-phosphomonoesterase and fungal phytase (bare= without cover crops, RT= reduced tillage, NT= no-till). The bars represent the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding models and F-Tests can be found in Table 1 and Supplementary Material S7.3*

Table 7.1 P-values for main effects and interactions of the fitted models of different P pools presented in Fig. 7.2. The factor levels were: cover crops (bare and cover crops), tillage (no-till and reduced tillage), date (Ferbuary and October) and depth (0-5 and 5-20 cm). The corresponding raw data can be found in Online Resource S2, models and full ANOVA tables in Online Resource S3, and the corresponding R code in Online Resource S4. Interactions where no significance was detected were omitted

	Variable					
Main effects and interactions	P_i	enzyme-stable P_{org}	enzyme-labile P_{org}	fungal phytase-labile P_{org}	monoesterase-labile P_{org}	diesterase-labile P_{org}
Cover crops (CC)	n.s.	n.s.	n.s.	**0.065**	n.s.	n.s.
Depth	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Date	**<0.001**	n.s.	n.s.	n.s.	n.s.	n.s.
Tillage	n.s.	n.s.	n.s.	**0.012**	n.s.	n.s.
CC x Depth					**0.042**	
CC x Date	n.s.	n.s.	**0.012**	n.s.	0.078	n.s.
Date x Depth	**0.045**	n.s.	n.s.	n.s.	n.s.	n.s.
CC x Tillage	n.s.	n.s.	n.s.	**0.015**	n.s.	n.s.
CC x Date x Depth	**0.011**	**0.05**	n.s.	n.s.	**0.024**	n.s.
CC x Tillage x Date x Depth	n.s.	**0.041**	n.s.	n.s.	n.s.	n.s.

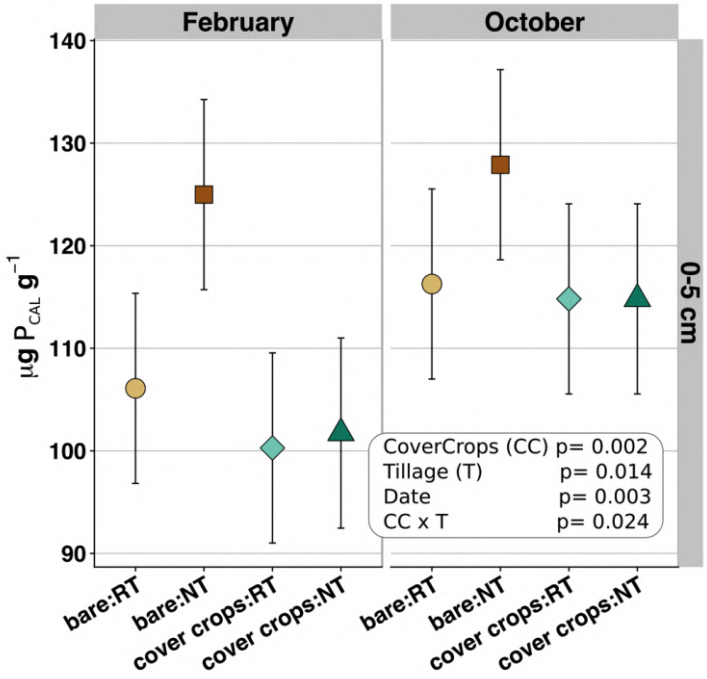


Figure 7.3 *Calcium acetate lactate extractable phosphate (P_{CAL}) under the different treatments at 0-5 cm (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 %. The corresponding model and F-test can be found in Supplementary Material S7.3*

Microbial carbon, microbial phosphorus and total PLFAs

Microbial carbon (C_{mic}) and total PLFA concentrations were used as proxies for microbial biomass. Cover cropping enhanced microbial biomass in the topsoil (Fig. 7.4 a and b) by around 12 %. After the growing season of soybean in October, microbial biomass increased compared to February. The measured P_{mic} in February was highest in the cover crop treatment with NT, but in October the plots with RT had higher P_{mic}, regardless of cover cropping (Fig. 7.4 c).

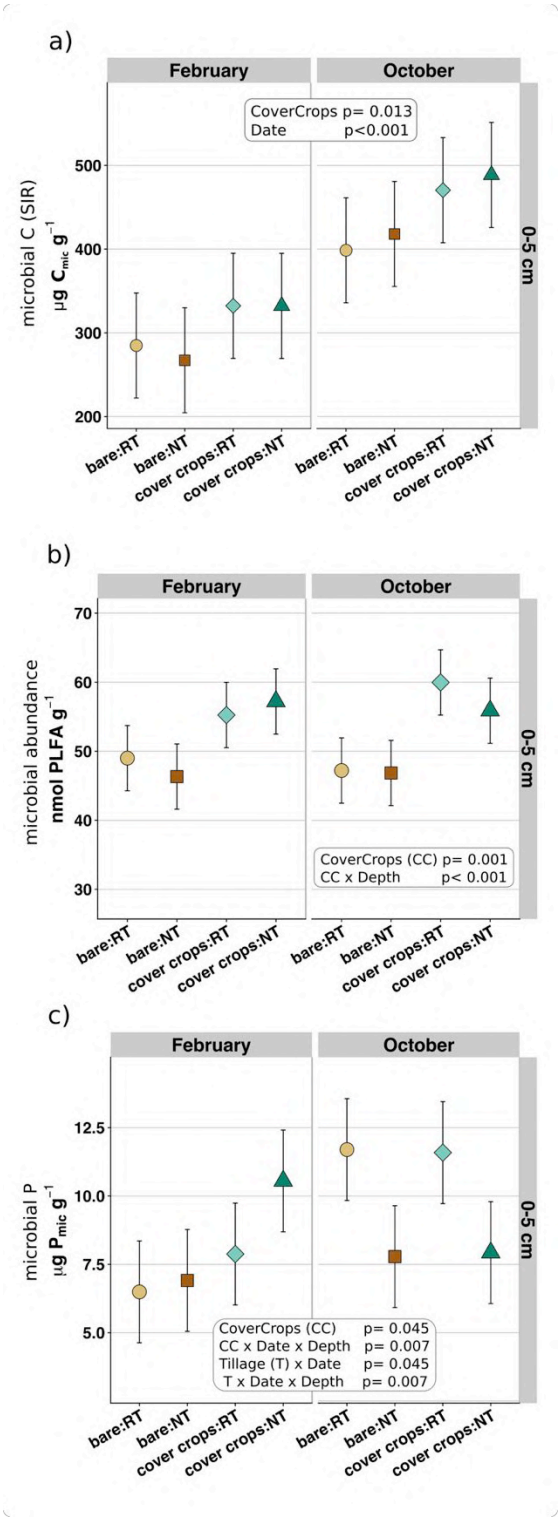


Figure 7.4 Microbial biomass: a) microbial C measured as substrate induced respiration (SIR) [µg microbial C g^{-1} soil], b) concentration of microbial PLFA biomarkers [nmol PLFA g^{-1}] and c) microbial P [µg P g^{-1}] by treatments at 0-5 cm (bare= without cover crops, RT= reduced tillage, NT= no-till, bare= without cover crops). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding models and F-Tests can be found in Supplementary Material S7.3

Microbial community structure (PLFA pattern)

Fatty acid biomarkers for Gram+ bacteria increased under cover crops (Fig. 7.5 a), whereas Gram- bacteria also increased in October under no-till (Fig. 7.5 b). Cover crops increased the abundance of fungal biomarkers, while reduced tillage showed a tendency toward further increase in comparison to no-till (Fig. 7.5 c). The abundance of AMF, based on the NLFA marker 16:1ω5, tended to increase under cover crops in the topsoil (Fig. 7.5 d). In the rooting zone (5-20 cm, Fig. S7.7) cover crops+NT had the highest content of 16:1ω5 NLFA in February. In general, the content of the mycorrhizal biomarker was higher at 5-20 cm and was more variable than in the topsoil, especially in October, after soybean growth.

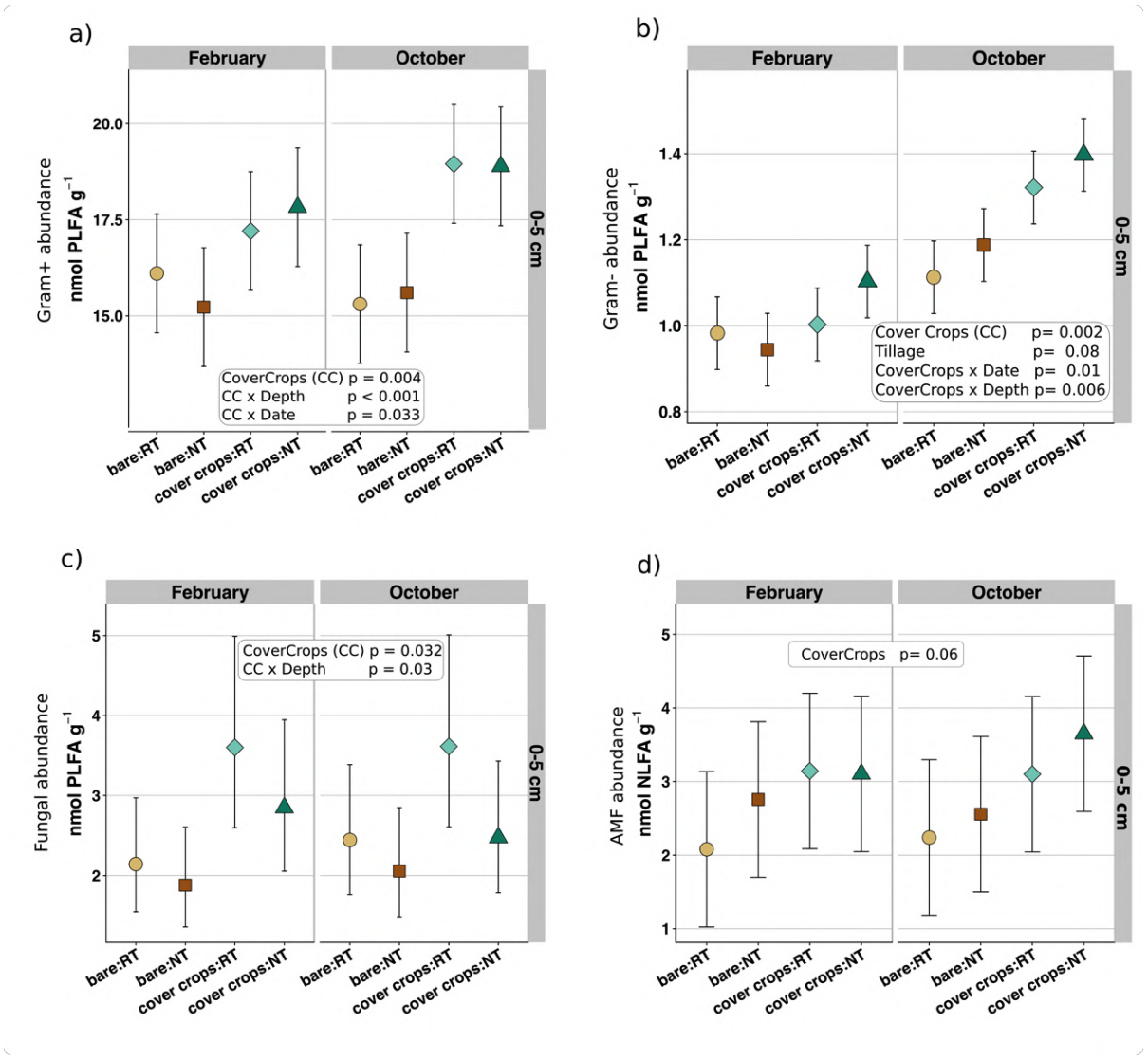


Figure 7.5 Concentration of fatty acid biomarkers of microbial groups: a) Gram+ [PLFAs i15:0, a15:0, i16:0, and i17:0], b) Gram- bacterial [PLFAs cy17:0 and cy19:0], c) general fungal [PLFA 18:2ω6,9 and 18:3ω6,9,12], and d) arbuscular mycorrhizal biomarkers [NLFA 16:1ω5] in nmol of fatty acids per gram dry soil under the different treatments at 0-5 cm (bare= without cover crops, RT= reduced tillage, NT= no-till, bare= without cover crops). Displayed are the estimated marginal means of the three field replicates; error bars indicate the modelled 95 % CI. The corresponding models and F-Tests can be found in Supplementary Material S7.3

Potential C- and P-cycling enzyme activities and metabolic diversity

Cover cropping increased the activities of phosphomonoesterases (Fig. 7.6 a), phosphodiesterases (Fig. 7.6 b), and β-glucosidases (Supplementary Material S7.3), especially in February and in combination with no-till. N-actyl-glucosaminidase activity was highly variable and did not exhibit any treatment effects (Supplementary Material S7.3). Cover crops also increased metabolic diversity, determined using a variety of C-substrates

calculated as average well colour development and Shannon-Weaver's diversity index from the carbon source utilisation data (Figs S7.7 and S7.8). The use of Glucose-1-Phosphate and DL-α-Glycerol Phosphate as carbon sources was increased by cover crops above average compared to the other C substrates (Figs S7.9 and S7.10).

When relating P-cycling enzymes with P pools in soils, the relation between enzymatic activity and enzyme-labile P pools was affected by the treatment (Fig. 7.7). Phosphomonoesterase activity, composed of phytases and other phosphomonoesterases, correlate negatively with the sum of the pools monoesterase- plus phytase-labile P_{org} in the topsoil in the no-till treatments (R^2=0.36, p=0.038, Fig. 7.7a), whereas with non-inversion tillage or in the lower 5-20 cm there was no visible relation at all. Conversely, the relation of phosphodiesterase activity with phosphodiesterase-labile P_{org} was not influenced by depth, but interacted with cover cropping and date, with a significant negative correlation with cover crops in February (R^2=0.43, p=0.041, Fig. 7.7b), but not later in the year in October.

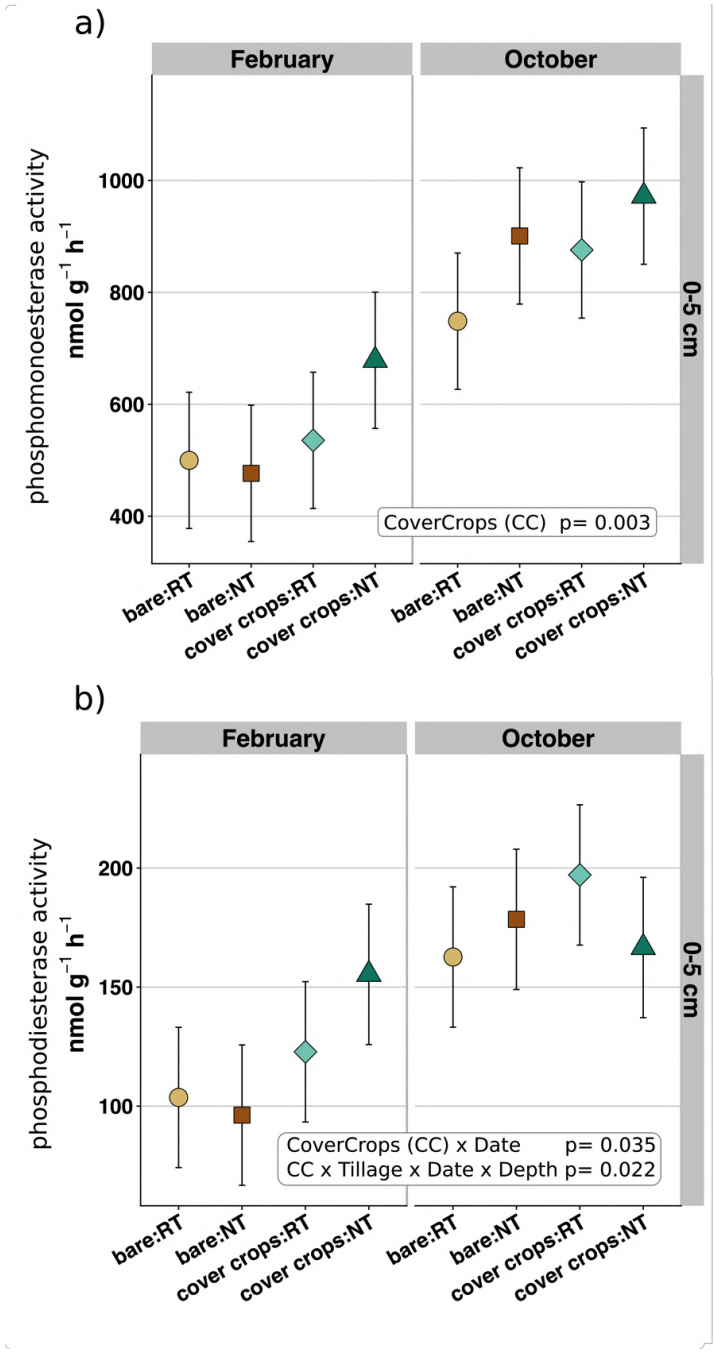


Figure 7.6 Potential activities of extracellular enzymes: a) phosphomonoesterase and b) phosphodiesterase in nmol of substrate per gram dry soil per hour under the different treatments at 0-5 cm (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding models and F-Tests can be found in Supplementary Material S7.3

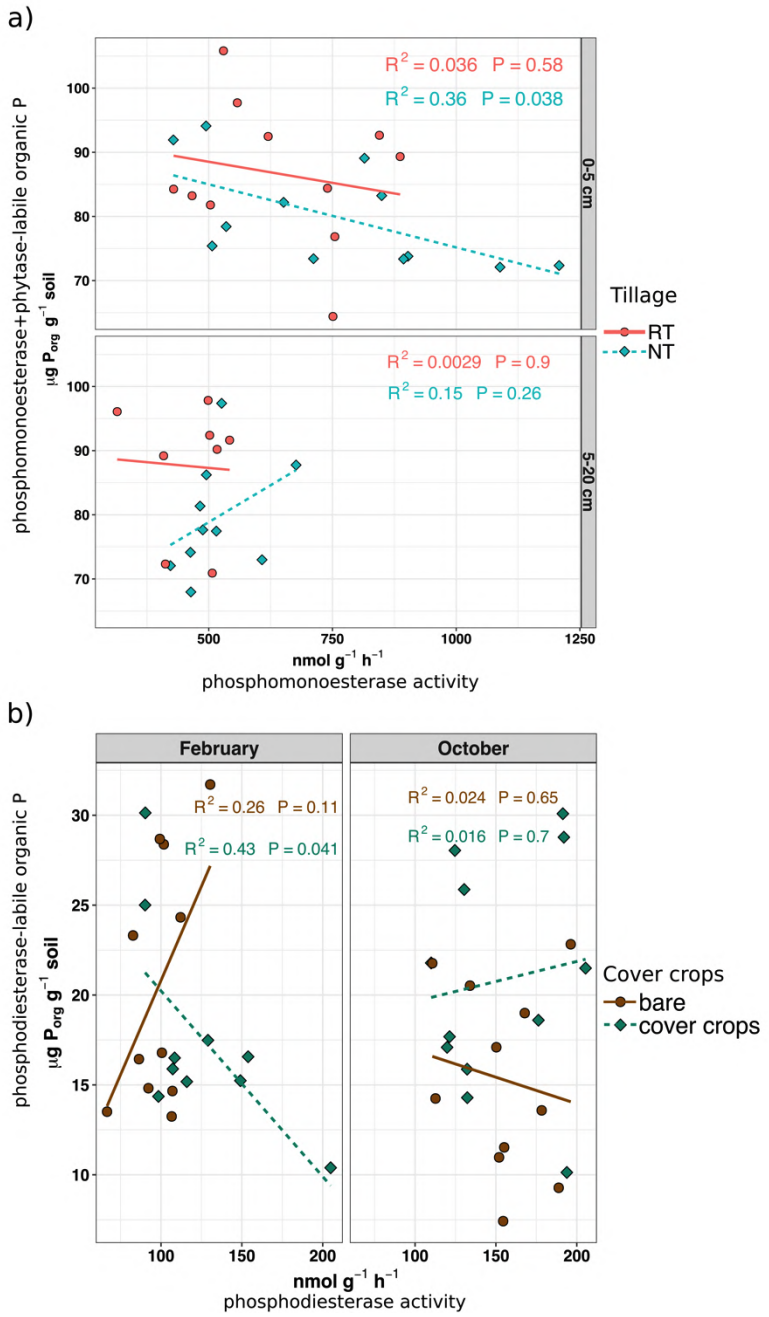


Figure 7.7 *Relation between enzymatic activity and the respective enzyme-available organic P pools for a) phosphomonoesterase and b) phosphodiesterase. The trend lines, R^2 and p-values were calculated using a simple linear model. As the relation of enzymatic activity and P_{org} pools interacted with depth and tillage as well as date and cover crops in the case of phosphomonoesterase and phosphodiesterase, respectively, the trendlines were fitted to the corresponding subsets. Coefficients and R-code can be found in Supplementary Material S7.3 and S7.4, respectively.*

Multivariate analyses of microbiological data

Linear discriminant analysis (LDA) was used to assess whether the treatments resulted in distinct microbial community structures and activity and to obtain an overview of the properties that dominated the dissociation (coefficients are reported in Supplementary Material S7.3).

Overall, the treatments resulted in differentiation of the soil microbial community structure and activity (Fig. 7.8). The effect of cover crops on community composition was most visible in October, indicated mainly by Gram+ and AMF biomarkers (Supplementary Material S7.3). Cover crops affected microbial activity already in February and the differentiation was dominated by enzymatic activities. Tillage had its greatest overall effect on microbial community structure and activity in October.

In this experiment, both phosphomonoesterase and -diesterase activity showed a positive correlation with the abundance of Gram+ bacteria (Pearson's R= 0.5 and 0.36; p=0.0002 and 0.012, Supplementary Material S7.3), Gram- bacteria (R=0.8 and 0.62; both p<0.0001), as well as fungi (R=0.4 and 0.37; p=0.003 and 0.008).

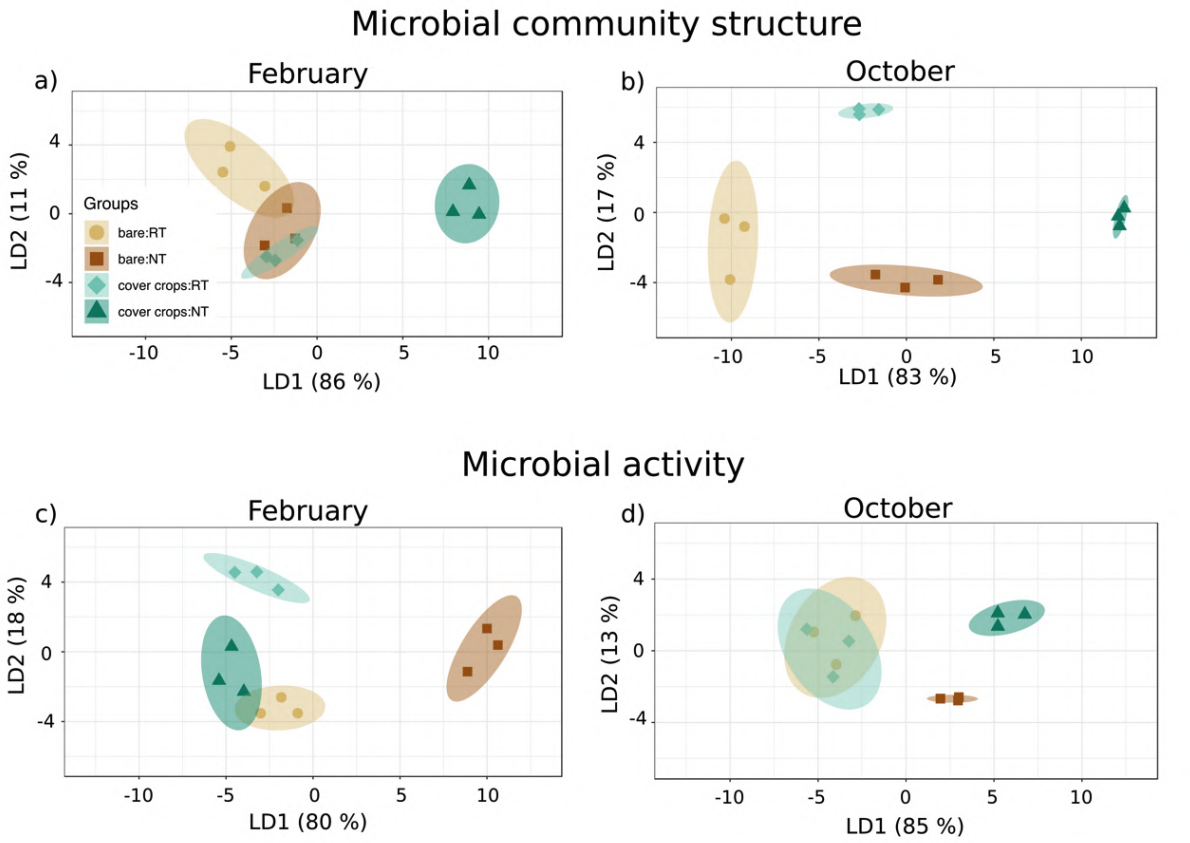


Figure 7.8 *Impact of cover crops (green= cover crops, brown= fallow) and tillage (light= reduced tillage/RT, dark= no-till/NT) on microbial community structure (a, b; fatty acid biomarkers) and activity (c, d; extracellular enzyme activity and substrate use capacity), in February (left) and October (right) at 0-5 cm, grouped by treatment. The parameters of each plot are summarised to a single point using linear discriminant analysis (LDA). The ellipses represent the 95 % CI of each group. Coefficients and R-code can be found in Supplementary Material S7.3 and S7.4, respectively*

7.5 Discussion

Cover crops influence P-cycling within soil-plant systems (Eichler-Löbermann et al. 2009; Honvault et al. 2020). In this study, combined chemical, biochemical, and microbiological methods were used to elucidate whether the growth of cover crops in combination with no-till might change microbial abundance and functions and lead to modifications in plant available P pools in soil. To interpret the data, first the impact of the treatments on soil P pools was characterised. Further, the role of the soil microbial community as a likely driver

for these changes is described and the mechanistic relationship of phosphatases to enzyme-available P_{org} pools are discussed. Then, the multivariate response of microbial activity and microbial community structure is outlined. Finally, the potential synergies between cover crops and no-till and the effects of the treatments on soil phosphorus dynamics are summarised.

Cover crops increase the enzymatic availability of organic P pools

The cultivation of cover crops induced a shift in P dynamics in the soil that could help to explain the commonly observed P benefit with cover crops (Hallama et al. 2019). The enzymatic availability of the P_{org} pools was sensitive to management practices (Fig. 7.2 b), despite the abundant P_i and P_{CAL} (Figs 7.2 a and 7.3) that dominated the P availability of the soil.

We suggest that the decrease in P_{CAL} (Fig. 7.3) was a result of both the uptake of P by cover crops, and by the immobilisation of P in the microbial biomass (Fig 7.4 c). This supports the concept that cover crops quickly take up labile P (Hallama et al. 2019) and that microbially-immobilised P contributes to the build-up of organic P in soil (Bünemann et al. 2008). Overall, our results indicate that the increased availability of enzyme-labile P in soil with cover crops (Fig. 7.2, Table 7.1) represents a relative shift from inorganic phosphate towards organic P sources, confirming our first hypothesis.

Conservation agriculture enhances the P-cycling capacity of the soil microbial community

As microbes are the main drivers of soil organic P dynamics (Richardson and Simpson 2011), the role of soil microorganisms underlying the observed shifts in labile P_{org} pools was investigated. The detected increases in enzyme-labile P_{org} pools with cover crops (Fig. 7.2) are concurrent with increases in microbial abundance (Figs 7.4 and 7.5, Supplementary Material S7.3) and activity (Fig. 7.6, Supplementary Material S7.3). The cover crop effect was, in most cases, greater than that of no-till, and treatment differences were more visible in the topsoil (0-5 cm) compared to the deeper soil layers (5-20 cm, data in Supplementary Material S7.2). Considerable treatment effects on microbial properties were already visible in February (Figs 7.4-7.6), whereas total enzyme-labile organic P increased in October. This

delayed response of the pools is logical if changes in the P pools are attributed to microbial activity. The observed cover crop effects may have resulted from the following processes: in February, the microbial community reflected mostly the direct effects of a living plant cover in the off-season (Kumar et al. 2013), although limited mineralisation of shoots and roots occurs during cold months (Kramer et al. 2013). In October, mineralisation of the cover crop biomass provided nutrients (Damon et al. 2014). Additionally, the rhizosphere of the soybean crop probably shaped the soil microbial community by rhizodeposition, altering the nutrient dynamics, as shown by Manna et al. (2007).

Not only changes in available P pools were of interest, but also in microbial drivers of these processes. The abundances of both Gram+ and Gram- bacteria increased under cover crops (Figs 7.5a and 7.b), probably due to above- and belowground litter inputs and rhizodeposits from cover crops. Tillage had no effect on Gram+, but NT tended to increase abundance of Gram-. This effect could be related to organic matter inputs from cover crops that favoured predominantly Gram- bacteria (e.g., copiotrophic Proteobacteria). The finding that Gram+ bacteria were less enriched in the conservation agriculture treatments could be explained by the fact that members of the biggest group of Gram+ bacteria in bulk soil, Actinobacteria, utilize predominantly more oligotrophic life strategies (Uksa et al. 2015; Ho et al. 2017). The finding that fungi benefited most from cover crops in combination with RT instead of no-till was unexpected, as fungi are commonly considered to be more sensitive to tillage than bacteria due to the disruption of their hyphal networks with soil movement (Jansa et al. 2003). We suggest that non-inversion tillage resulted in an increase in the abundance of saprotrophic fungi with RT (Fig. 7.5c), because of the availability of substrate due to the mixture of cover crop litter with the soil.

Increases in the activities of P cycling enzymes in cover crops+NT compared to the other treatments in February, were detected both in absolute values (Fig. 7.6) and per unit C_{mic}. The contributions of the different microbial groups to this increase were presumably unequal. Relating activities of P cycling enzymes to different groups of microorganisms showed that phosphomono- and -diesterase activities correlated positively with abundances of bacteria and fungi. The genetic potential for the production of acid and alkaline phosphatases is widespread in soil microorganisms (Bergkemper et al. 2016), but there are

no detailed studies of the abundance of single bacterial and/or fungal species‘ connections to *in-situ* activity of phosphatases. In our experiment, mainly bacteria might have increased the release of phosphatases to cover their demand for phosphate, while increasing microbial P immobilisation.

In order to evaluate enzymes from the same family but produced by different groups of soil microorganisms (Menezes-Blackburn et al. 2013), a commercial bacterial phytase was included in addition to the fungally-derived phytase in the enzyme addition assay. The bacteria-derived phytase mineralised around 20 % more P_{org} than the phytase derived from fungi. However, the addition of the bacterial phytase had more variable results (Fig. S7.5). The different amounts of phosphate released by bacterial and fungal phytases indicate that the two enzyme families act on different but overlapping subpools of P_{org} (Hill and Richardson 2007). Apparently, the differences in terms of enzyme activity between the two phytases produced by these organisms may reside more in the environmental conditions (i.e., pH) of their location (Wyss et al. 1999) than on substrate specificity. Fungal phytase-labile P was especially abundant in cover crops with reduced tillage (Fig. 7.2b), corresponding to the greatest fungal abundance (Fig. 7.5c). Therefore, it seems reasonable that phytate produced by fungal microorganisms (Turner 2007) contributed to the pool of fungal phytase-available P_{org}, representing a substrate that is located in micro-environments with favourable conditions for the activity of fungal phytases.

Arbuscular mycorrhizal fungi are of particular interest in plant production due to their role in P nutrition for many crops, and enhanced AMF abundance after cover crops is positively related to phosphorus uptake (White and Weil 2010). In our experiment, the abundance of AMF biomarker NLFA 16:1ω5 tended to be greater under cover crops (Fig. 7.5d), but tillage had apparently no effect on AMF. Possible explanations for the lack of an AMF abundance response to no-till could be that the dominant AMF species were resistant to tillage effects (Jansa et al. 2003) or to antagonistic relationships between different soil microorganisms (Li et al. 2020). Overall, our results add to the emerging body of literature that has shown the evident and positive effects of cover crops on microbial properties (Kim et al. 2020a) and relate these changes in microbial properties with soil P dynamics,

potentially increasing labile organic P pools. The effects of cover crops were more evident than those of no-till.

Organic P compounds and phosphatase enzymes

The approach of quantifying soil P_{org} pools according to their potential hydrolysability by adding substrate specific enzymes (phytase, phosphomonoesterase and phosphodiesterase) together with the assessment of soil enzymatic activity (phosphomonoesterase and phosphodiesterase activity) provides deeper insights into the dynamics of P_{org} cycling than have before been seen. The EAA method uses excess enzyme concentrations to measure the potential availability of different native P_{org} pools for enzymatic mineralisation, while methods analysing enzyme activities optimise the concentrations of P_{org} substrates to assess the amount of enzymes in the soil, i.e., the mineralisation potential of organic compounds.

The association of monoesters and diesters, two of the most abundant chemical forms of P_{org}, with their respective enzymes, appeared to be influenced by the treatment. To interpret these findings, we must keep in mind the different processes that control the substrate-enzyme relation, as they affect each other mutually (Bünemann et al. 2011). The production and release of phosphatases by roots and microorganisms in soils is assumed to be controlled mainly by the requirements of the organisms and the concentration of available substrate (Quiquampoix and Mousain 2005). However, other factors, such as stabilisation and turnover times of P-cycling enzymes, as well as complexation of substrates, seem to be important for enzymatic turnover *in-situ* (Rao et al. 2000). The other side is the size of available substrate pools. Here, monoesters (including inositol-P) constitute most (in our case, around 80 %, Fig. 7.2b) of the enzyme-labile P_{org}, although chemical stability and sorption on particle surfaces limit their availability for mineralisation (Gerke 2015). Diesters, on the other hand, interact less with the soil matrix, but persist to a certain degree because of the low stability of the enzymes that degrade them (Lang et al. 2017; Jarosch et al. 2019; Müller et al. 2020). Counter-intuitively, phosphodiesterase activity may constitute a rate-limiting step for mineralisation in a soil with P_{org} pools formed by abundant but enzymatically unavailable monoesters and less abundant, but more available diesters (Turner and Haygarth 2005). The absence of a clear main effect of enzymatic activity as a

covariate for ezyme-labile P_{org} may indicate that the soil was not in a steady-state, where enzymatic activity and organic P control each other mutually. Both enzymatic activity and organic P pools varied over time and depth and were affected by the addition and availability of fresh organic matter and microbial activity.

The detected increases in phosphomonoesterase activity with cover crops are accompanied by an increased capacity of the microbial community to use specific phosphate-bearing substrates, such as glycerol-phosphate and glucose-1-phosphate (Figs S7.9 and S7.10). Besides a general increase in organic compounds and microbial mineralisation under cover crops, one explanation for this specific increase in the capacity to degrade phosphate-bearing substrates could be the presence of phosphate compounds in root exudates of cover crops and the adaptation of microbes to use these substrates effectively. Sugar phosphates are involved in intracellular carbohydrate metabolism and participate in co-transportation of plastid-localized sugar-phosphate in several species of plants (Flügge et al. 2011). Although import and export mechanisms of sugar phosphates into and from root cells are not characterized, these compounds are detected in plant exudates (Sasse et al. 2018). In addition, cover crops induce priming effects in the rhizosphere by influencing the turnover of soil organic matter (Dijkstra et al. 2013), hence altering soil nutrient content, including phosphorus. However, higher turnover of glycerol-phosphate and glucose-1-phosphate could alternatively reflect the higher demand for P when microbial biomass is increased under cover crop treatment. Therefore, stimulation of phosphomonoesterase activity becomes plausible. Unfortunately, the biolog plates used in this study did not contain any substrates with phosphodiesters.

The enhancement of enzyme activity under cover cropping can be explained by the increase in the availability of organic P substrates (Quiquampoix and Mousain 2005), the reduction of the concentration of P_i (i.e., product-inhibition) (Burns and Dick 2002), and the increase in microbial abundance, as well as microbial production of phosphatases. With the detection of an association between the increase in abundance of various microbial groups, increased enzymatic activity and increased enzyme-available P_{org} pools, we confirm our second hypothesis, which assumed that a stimulated microbial community with enhanced functions would be associated with changed P pools. With our current understanding of soil

organic P dynamics, the changes in P_{org} pools can be expected to be driven by the stimulation of the microbial community (Richardson and Simpson 2011). However, simultaneous substrate-driven processes, e.g., increases in microbial activity due to greater availability of P_{org} from cover crops residues, may also take place.

Multivariate response of microbial functions and microbial community composition to conservation agriculture

The soil microbial community was affected by the conservation agriculture treatments, resulting in a differentiated community structure and activity (Fig. 7.8). In February, the treatments, especially cover cropping, affected microbial activity more than community structure, which is in line with other studies that have found microbial activity to be more sensitive than community composition to management changes (Bier et al. 2015). By October, both tillage and cover crops had resulted in distinct community compositions, though for microbial activity tillage was more important. The tillage operations in the RT treatments that were done after the sampling in spring likely were the reasons for the greater tillage effect in October.

Functional diversity, calculated as Shannon-Weaver's H from carbon substrate group utilisation, increased under cover crops and NT (Figs S7.7 and S7.8). It is commonly reported that cover crop mixtures increase microbial diversity (Kim et al. 2020a). In addition, tillage reduction may increase or preserve spatial heterogeneity that would be destroyed due to homogenisation by tillage. Besides potential pathogen suppressing effects (Weller et al. 2002), a diverse community with a variety of nutrient acquisition strategies may have an advantage for the utilisation of different nutrient pools, leading to their increased availability to the community as a whole. This theory of resource partitioning also applies to organic P pools (Turner 2008). The characterisation of cover crops according to plant traits provides a promising approach to understand the cover crop effects on soil microbes and hence P availability (Wendling et al. 2016; Boeddinghaus et al. 2019). This perspective, applied to plant communities in the form of commuity mean traits (Garnier et al. 2007), could also help to predict the complex action of cover crop mixtures.

Conservation agriculture techniques: synergy between cover crops and tillage reduction?

Cover crops and no-till are two techniques in agricultural management that are often used with the expectation of enhancing microbial abundance and activity, and consequently crop nutrition. Substrate inputs and protection by the living and dead cover crops sustain the soil biota (Mukumbareza et al. 2015). No-till increases soil heterogeneity both in the soil profile and at the aggregate scale, with profound impacts on the soil microbial community (Young and Ritz 2000). This in turn provides greater more opportunity for soil to rest and a concentration of nutrients and soil organic matter (SOM) at the surface (Kabiri et al. 2016). A synergy between both management techniques is often assumed and frequently found (Wittwer et al. 2017; Boselli et al. 2020), but there are also reports of a greater relative improvement of in microbial properties in under tillage treatments (Balota et al. 2014).

Particularly to make the comparison between the no-till and reduced tillage, soil samples were taken at two different soil depths. The treatment effects in the deeper soil layer (5-20 cm) were generally rather weak; this was the case both for cover crops and for tillage. One factor could be the chosen sampling depth: the tillage operations in RT were conducted only up to 10 cm soil depth, in some cases even less (Supplementary Material S7.1). Thus, cores taken at the 5-20 cm depth included some soil that was not affected directly by the tillage treatments. However, the concentration of the cover crop effects at the surface corresponds to litter placement of aboveground plant biomass from crops and cover crops, and we had expected also effects of cover crop roots and their exudates at the 5-20 cm depth (Austin et al. 2017; Schmidt et al. 2018).

In our experiment, judging only by the results of the plots without cover crops, the positive effects of no-till on soil properties were rather limited. However, when comparing reduced tillage and no-till in the plots with cover crops, the picture gets more complicated. Fungal phytase-labile P_{org} (Fig. 7.2b) was greatest with cover crops and reduced tillage, while other properties, such as abundance of Gram- bacteria or phosphomonoesterase and phosphodiesterase activity in February (Figs 7.3 and 7.6) showed synergistic effects of the combination of cover crops with NT. Despite observed shifts in both microbial community composition and activity (Fig. 7.8), it is not possible to judge these differences in terms of agronomical relevance easily. We are still missing some of the causal relationships between

the different soil and plant P pools, microbial community structure, and their potential functions (George et al. 2018). Therefore, our third hypothesis about synergistic effects of cover crops and no-till on soil microbial properties and P-cycling capacity can be only partially confirmed. Further experiments, taking into account the influence of conventional management (Romdhane et al. 2019) and alternative management systems (Mulvaney et al. 2017) are necessary.

In summary, assessment of the treatment combinations revealed a clear enhancement in microbial abundance and activity under cover crops compared to bare fallow (Fig. 7.9). This potential for (micro-) biological P cycling came with an increase in organic P pools. However, available inorganic P (here measured as P_{CAL}) was greatest in the bare fallow treatments.

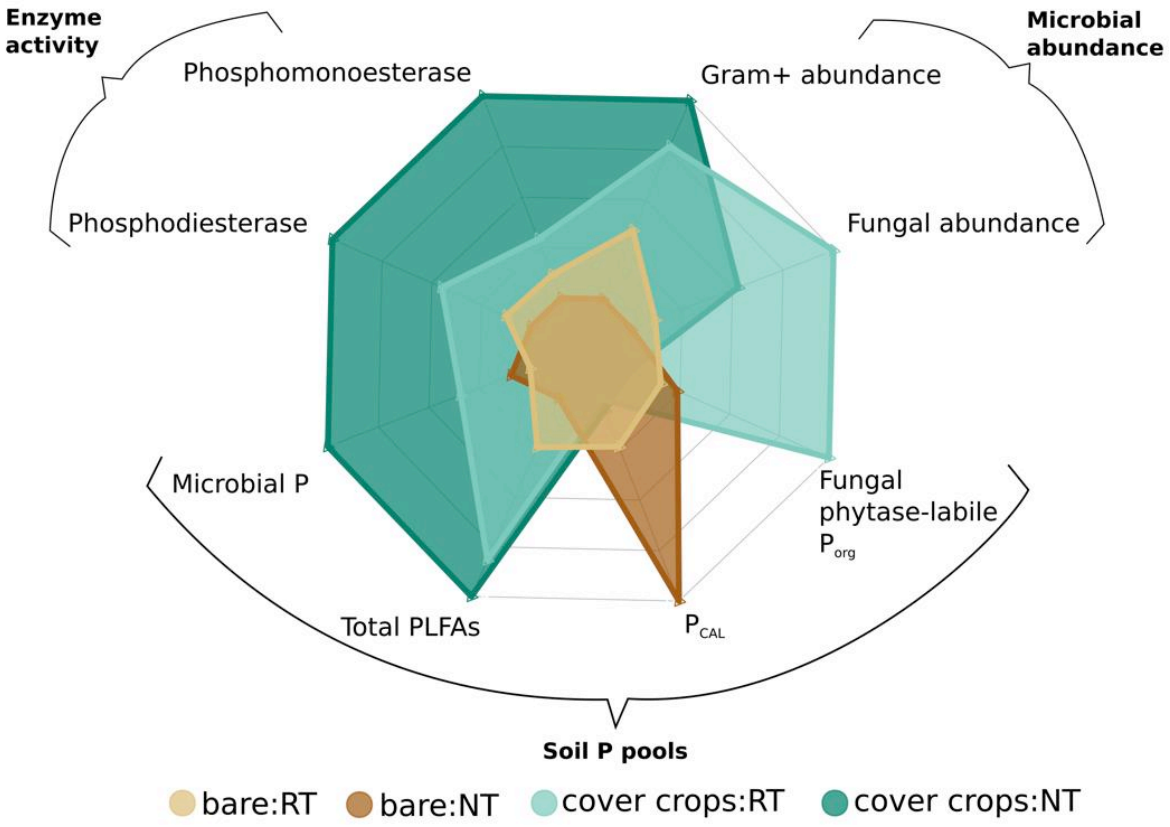


Figure 7.9 *Radar chart summarizing the effects the four treatments of the experiments (bare vs cover crops and reduced tillage vs no-till), on several soil phosphorus pools and microbial P-cycling in February at 0-5 cm. The variables represent (clockwise from the top right): Microbial abundance (Gram+ and fungal abundance); Soil P pools (fungal phytase-labile organic P (P_{org}), calcium-acetate-lactate extractable P (P_{CAL}), total Phospholipids (PLFAs), microbial biomass phosphorus; and enzyme activity (phosphodiesterase and phosphomonoesterase). Grid lines correspond to the 0, 25, 50, 75, and 100-quantiles of each variable over all dates and depths (R-code can be found in Supplementary Material S7.4)*

7.6 Conclusions

This study demonstrated that a cover crop mixture and no-till, as components of conservation agriculture, could enhance soil microbial abundance and activity and change the phosphorus dynamics in a temperate agricultural soil by stimulating organic P cycling. Cover cropping in particular shifted organic P towards pools of higher potential availability for enzymatic hydrolysis. Soil microbial abundance and activity were related to changes in P pools, highlighting the importance of soil microbes for nutrient cycling. More research is needed to study the drivers of the relation between enzymatic activity and organic P pools.

Despite the fact that this experiment was conducted in a field where P availability was not a limiting factor, the system responded after only two seasons of cover cropping. In the bare fallow treatments, representing more conventional systems without cover crops, P dynamics appear to have been dominated by the abundant available inorganic P. Although this study represent only one site and has to be repeated for more sites, we elucidated these two distinct patterns that might explain why both systems work in practice on many farms in central Europe: On the one hand, the conventional input-based, yield-optimised approach with a lower complexity; and on the other hand, the concept of sustainable intensification, making use of biological functions and internal nutrient cycling.

Cover crops are an important tool to mine P from the soil and hence to reduce the necessity to apply P as a mineral fertilizer. Tillage reduction also appears to have an impact, but the agroecosystem might need a longer time for a new measurable equilibrium to be achieved. These two components of conservation agriculture can help to reduce the current high consumption of P fertilizers and to decrease the environmental impact of agriculture. Cover crops constitute a promising, multifunctional tool for sustainable intensification of agriculture, provided species selection and management match the agricultural goals. Scientific efforts and agricultural policies should be directed to overcoming barriers to the widespread adoption of these soil improving cropping systems.

7.7 Acknowledgements

We thank Doreen Berner for assistance in the laboratory, Ines Binder for the soil profile classification, Markus Rodehutscord for provision of the bacterial phytase and phytate

model substrates, Andy Neal for his input to the discussion and Hans-Peter Piepho for his statistical support.

Moritz Hallama received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 677407 (SoilCare project). The field experiment is part of the project *Konservierender Ackerbau,* funded by the Ministerium für Ländlichen Raum und Verbraucherschutz Baden-Württemberg.

7.8 Supplementary Material

Supplementary Material S7.1 *Spreadsheet (.xlsx) with the agronomic management. Available at the public repository Open Science Framework (https://osf.io/m9d5c/)*

Supplementary Material S7.2 *Spreadsheet (.xlsx) with the full dataset. Available at the public repository Open Science Framework (https://osf.io/m9d5c/)*

Supplementary Material S7.3 *Document (pdf) with fitted models and results of ANOVA. Available at the public repository Open Science Framework (https://osf.io/m9d5c/)*

Supplementary Material S7.4 *R-code (.Rmd) used for statistical analysis and elaboration of figures. Available at the public repository Open Science Framework (https://osf.io/m9d5c/)*

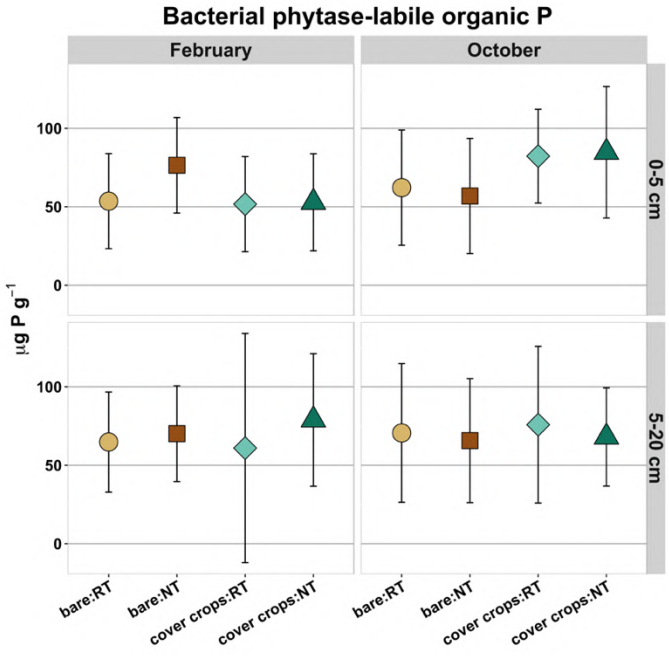


Figure S7.5 *Organic P hydrolysed by added bacterial phytase under the different treatments (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding model and F-test can be found in Supplementary Material S7.3*

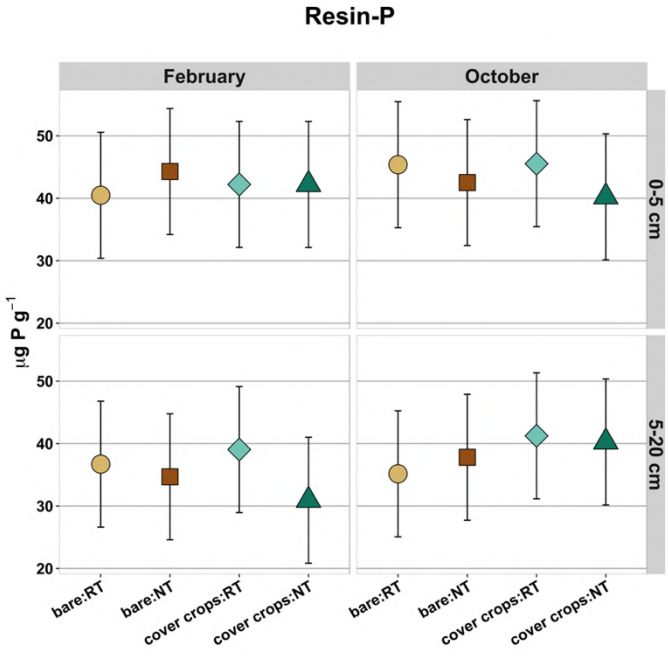


Figure S7.6 *Resin-P (P_{resin}) in soil-water-extract under the different treatments (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding model and F-test can be found in Supplementary Material S7.3*

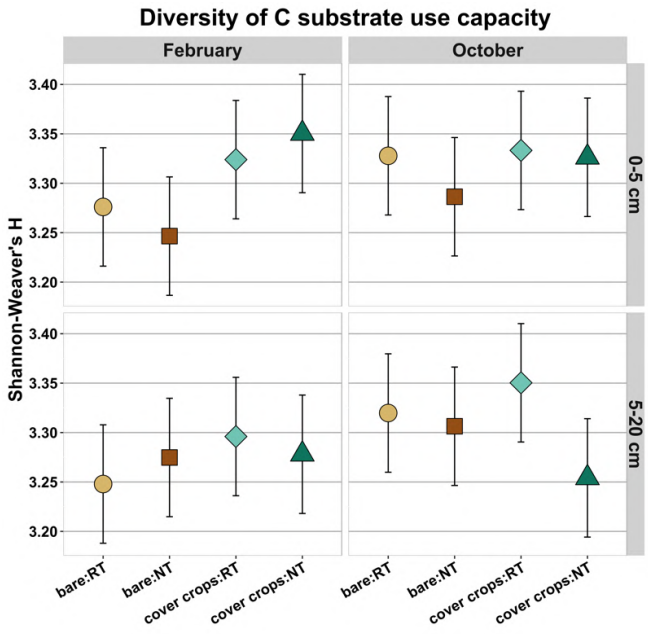


Figure S7.7 *Shannon-Weaver's diversity index of carbon substrate use capacity under the different treatments (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding model and F-test can be found in Supplementary Material S7.3*

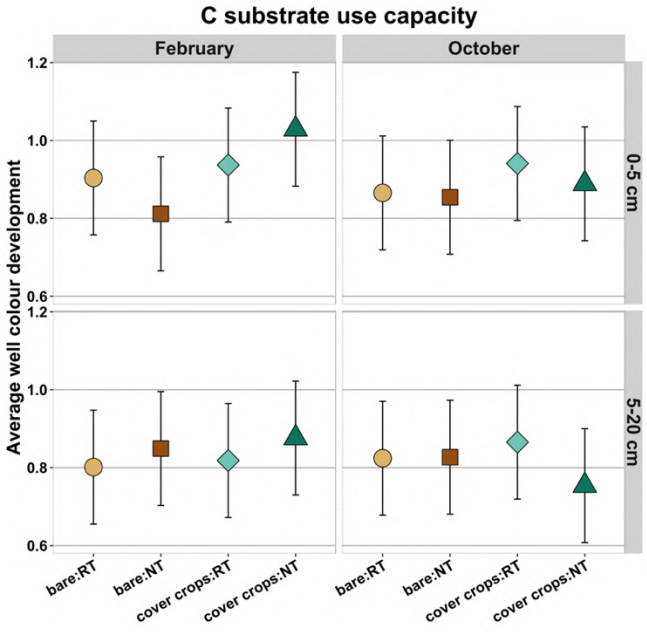


Figure S7.8 *Average well color development (AWCD) of carbon substrate use capacity under the different treatments (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding model and F-test can be found in Supplementary Material S7.3*

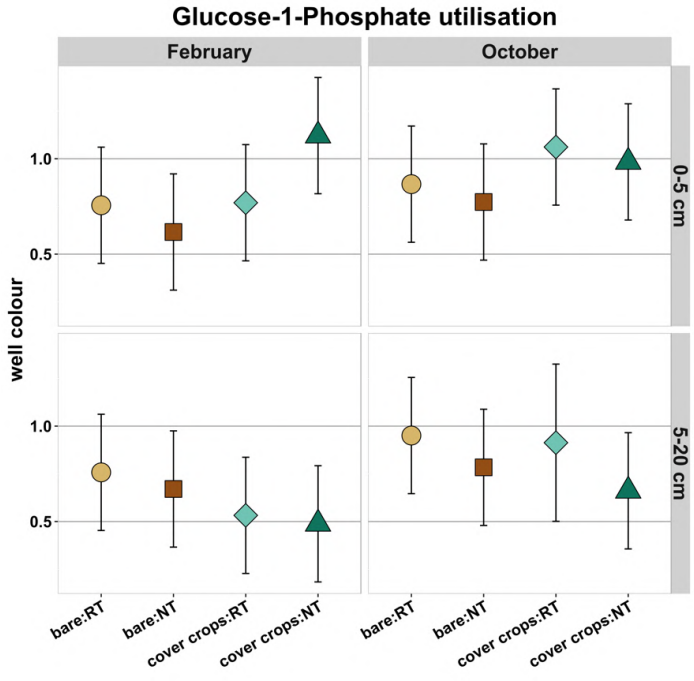


Figure S7.9 *Capacity of the soil microbial community to use Glucose-1-Phosphate as a carbon source under the different treatments (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding model and F-test can be found in Supplementary Material S7.3*

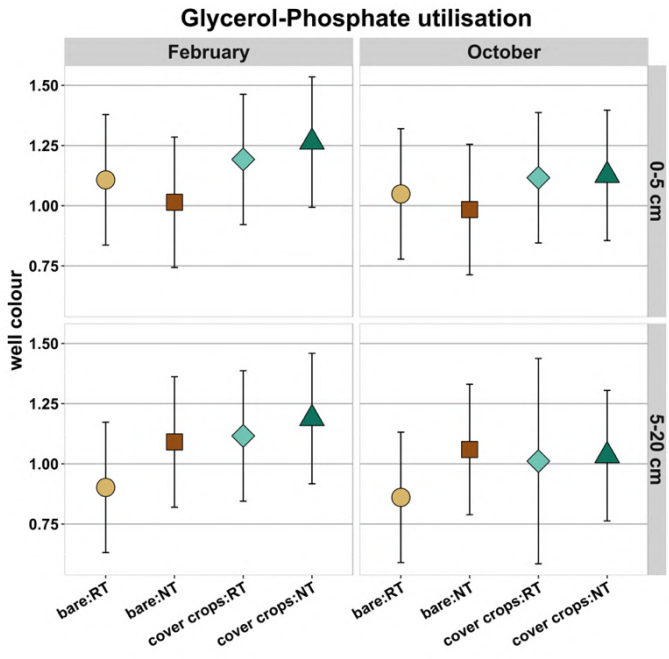


Figure S7.10 *Capacity of the soil microbial community to use Glycerol-Phosphate as a carbon source under the different treatments (bare= without cover crops, RT= reduced tillage, NT= no-till). Displayed are the estimated marginal means of the three field replicates; error bars show the modelled 95 % CI. The corresponding model and F-test can be found in Supplementary Material S7.3*

8 General discussion

The studies presented in this dissertation provide evidence that cover crops constitute a promising multifunctional tool for more sustainable management of P in agriculture. Although there is still a long way to reach the estimated potential of 25 % of agricultural cropland with cover crops (Poeplau and Don 2015), adoption of this technique by farmers has increased greatly over the last decade, while at the same time, agricultural policies to support it are gaining *momentum* in many countries (Kanter and Brownlie 2019). The hazards of legacy P and the opportunities for its management are on the agendas of the Sustainable Phosphorus Alliance located in the USA and the European Sustainable Phosphorus Platform.

8.1 How do cover crops increase the access of P to main crops?

In this dissertation, a number of properties related to P-cycling and the microbial community were analyzed. With the resulting data we are now able to discuss and evaluate specific aspects of the proposed conceptual framework of cover crop-derived P benefits (Fig. 8.1). The three pathways of P benefit presented above (*plant biomass*, *biochemical modification,* and *soil microbial enhancement*) have varying degrees of relative importance in different agricultural contexts, utilizing distinct mechanisms and responding to controlling factors. In the following, we discuss how the results of our studies add to the general knowledge of the discipline and the extent to which they answer the research questions outlined in the Introduction.

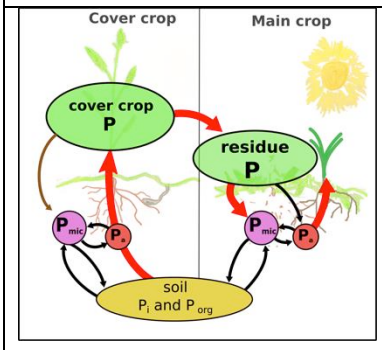
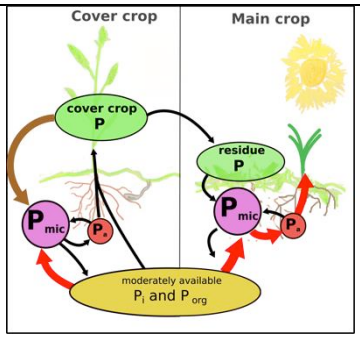
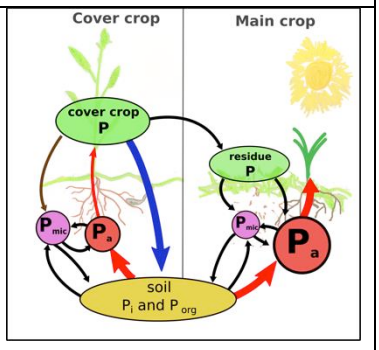
Pathways of P benefit		
Plant biomass	*Microbial enhancement*	*Biochemical modification*
		
Assessed properties		
Cover crop biomass P	PLFA/NLFA Enzyme activity P_{mic} Enzyme-labile P_{org} *phoD*	P_{resin} $P_{sorption}$

Figure 8.1: The three proposed pathways of cover crop-derived P benefit to the main crop. Arrows in red illustrate the main movements of P, brown arrow represents the input of rhizodeposits and blue arrow the exudation of carboxylates that mobilize soil P of limited availability. *Left*: Via the *plant biomass pathway*, P is taken up and stored in the cover crop biomass. During litter decomposition the released P is potentially available to the main crop; *center*: Via the *microbial enhancement pathway*, cover crop's rhizodeposition (brown arrow) increases microbial abundance and activity, facilitating access to enzyme-labile P_{org} for the main crop; *right*: Some cover crops perform a *biochemical modification* of their rhizosphere, increasing the availability of phosphate to the main crop. P_{mic} = microbial biomass; P_a = available phosphate; P_i = inorganic P pools; P_{org} = organic P pools

In our experiments, cover crop biomass contained up to 25 kg P ha^{-1} that was released after growth termination during litter mineralization (Fig. 6.2). This quantity could have covered the totality of the main crops' P requirements, underscoring the potential of the *plant biomass pathway* (Fig. 8.1 *left*). These results notwithstanding, even in situations with such an exceptionally high cover crop biomass, the assumption that all of the contained P is readily available for the main crop falters somewhat, since the time between cover crop termination and main crop establishment is often too long for the main crop to take advantage of available P. Most of the nutrients contained in cover crop litter are transferred to the soil in a matter of several weeks, not months (Damon et al. 2014). In contrast, the

highest total nutrient uptake of crops usually takes place in the grain filling stage, although earlier developmental stages can also be critical for yields (Grant et al. 2001; Pedersen et al. 2021). This time lag makes it unlikely that a main crop can access a substantial part of the litter-derived P directly from the pool of available phosphate, although some more complex forms of P_{org} might be released more slowly. Most litter-derived P reaches the main crop after one or several cycles of incorporation and release via soil microbial biomass or other P pools (Bünemann et al. 2012). Carbon and other nutrients contained in the plant litter provide a good foundation for increasing microbial biomass and activity, affecting the availability of various inorganic and organic pools. Therefore, in the case of the *plant biomass pathway*, soil microbes may be pivotal in P-cycling.

As mentioned above, some plant species use as their P acquisition strategy a particularly intensive modification of the soil chemistry in their rhizosphere, directly reducing the sorption capacity of P. With cover crops acting via this *biochemical modification pathway*, increased availability of phosphate could benefit the main crop (Fig 8.1 *right*). Nonetheless, although in Study #2 we detected a trend towards decreased P sorption capacity in the rhizosheaths of the cover crops (Fig. S6.7), the concentration of available phosphate under the main crop, measured as P_{resin}, was not affected by cover cropping (Fig. S6.5). The absence of a detectable cover crop effect related to this pathway may be related to the choice of crop rotations. On one hand, in the meta-analysis of Study #1, this pathway was only found when *Lupinus* sp. was used as a cover crop. It is possible that some other cover crop species simply do not act via this mechanism. On the other hand, in both studies, soybean, a member of the Fabaceae family with its own considerable capacity to access less available P pools by exudation of carboxylates, was used as main crop (Maltais-Landry 2015). Although in our studies we did not measure carboxylate exudation, the observation of increased P_{resin} in the rhizosheaths of soybean compared to the surrounding bulk soil points in this direction (Fig S6.5).

In both of the pathways discussed above, the choice of cover crop plant plays a major role. However, these pathways do not take into account the important role of P_{org} to P-cycling in terrestrial ecosystems (George et al. 2018) and the potential of access to legacy P_{org} by cover crops to improve plant nutrition. As microbes play a key role in the cycling of

P_{org}, one of the main objectives of this dissertation was to elucidate specifically how complex plant-soil-microbe interactions are affected by cover crops and whether the *soil microbial enhancement pathway* increases the availability of P_{org}.

The positive effects of cover crops on the soil microbial community were highly significant, both in the studies consulted in the meta-analysis as well as in our own field experiments. Microbial abundance increased in cover cropped plots compared to bare fallow treatments (Figs 6.5. and 7.4), as did the activity of P-cycling enzymes (Figs 6.6 and 7.6). Microbial community structure was also affected, and analysis of fatty acid biomarkers revealed that microbial groups benefitted from cover crops in the order saprotrophic fungi > gram-negative bacteria > gram-positive bacteria > AMF (Figs 6.5 and 7.5). Regarding the effects on microbial groups, we consider two aspects especially noteworthy, both related to the fungal kingdom: we observed large and lasting increases in the abundance of saprotrophic fungi, but surprisingly limited effects on AMF.

The disproportional increase in abundances of soil fungi with cover crops resulted in increased fungal:bacterial ratios. As conventional agricultural ecosystems are usually bacteria-dominated, management techniques that favour fungi might improve agroecosystem functions (Frey et al. 1999), including C storage (Six et al. 2006) and nutrient mobilisation (Ceci et al. 2018). Cover cropping has also been shown by other studies to enhance fungal abundance (Benitez et al. 2016). Historically, the degradation of labile organic matter has been considered the work of bacteria, although more recent studies indicate that fungi also participate in the early phases of litter decomposition (Kramer et al. 2012). Cover crop litter used as an organic amendment in a controlled pot experiment increased fungal abundance while reducing potential pathogens (Clocchiatti et al. 2020). In the aforementioned study, the effects of cover crop litter amendment were rather transient, especially favouring members of the phylum Mortierellomycota (the so-called *sugar fungi*), while in our own experiments lasting increases in fungal abundance were observed. Further studies are necessary to evaluate the effects of cover crops on the composition of fungal community composition and its functions.

It is frequently claimed that cover cropping increases the abundance of AMF, providing a benefit for main crops that depend on this symbiosis for pathogen control (Turrini et al.

2016) or P acquisition (White and Weil 2010). Previous work also demonstrated that high P availability supresses the formation of mycorrhizae (Thomson et al. 1986). However, the results of our field experiments regarding the cover crop effect on mycorrhizal fungi are ambiguous. In Study #2, conducted in a soil low in available P and with a high initial AMF abundance, the effect of the presence of cover crops on AMF lipid abundance was inconclusive (data in Supplementary Material S6.1), despite the fact that the cover crops tested represent plant families with drastically different degrees of mycorrhization (phacelia are highly mycorrhizal, while buckwheat and mustard are non-mycorrhizal). The concentration of the storage lipid NLFA 16:1ω5 was lower in the rhizosheath than in the bulk soil, but there was no effect of cover crop species. In contrast, the PLFA 16:1ω5, a constituent of the cell membranes of mycorrhizal fungi, was greater in the rhizosheaths of cover crops compared to the surrounding bulk soil. The consistently observed differences in the abundance of cell membrane lipids and storage lipids can be interpreted as representing spatially distinct foraging strategies, with a more active and dense hyphal network in the rhizosheath, and an established hyphal network with a storage function in the bulk soil (Gavito and Olsson 2003). However, in this case we would again anticipate detectable differences among mycorrhizal and non-mycorrhizal cover crop species. Additionally hampering our interpretation is the fact that the PLFA 16:1ω5 is also present in some other microorganisms (Ngosong et al. 2012) and the two types of fatty acids have distinct turnover times; PLFAs are used by microbes as P source, while NLFAs degrade more slowly (Bååth 2003). In Study #3, conducted in a soil with a relatively high concentration of available phosphate due to earlier mineral fertilization, AMF abundance tended to be positively affected by cover cropping (Fig 7.5). There was no observed increase due to tillage reduction in the no-till treatments, although other studies report that tillage affects AMF abundance negatively (Kabir 2005). Based on fatty acid biomarkers, our results indicated, therefore, that the effect of a cover crop on AMF depended mainly on the initial abundance of these fungi and was apparently unaffected by P availability and tillage.

Plants of phylogenetically distant families have been shown to differ in their associated rhizobiomes and their effects on the soil microbial community (Maul and Drinkwater 2010). These findings notwithstanding, results of the field experiment of Study #2 did not confirm

this, as the effects of different plant species were less pronounced than expected, especially for AMF (Fig 7.5). It could be that the field experiment was conducted in a soil with existing quite high microbial abundance and activity, or due to the presence of weeds. Nonetheless, it must be kept in mind that the effects of cover crop species and plant biomass produced are prone to be confounded, especially when using broad methods such as PLFAs. Cover crops that produce high biomass will have also greater effects on the abundances of various soil microbial groups, as root exudation depends on the overall photosynthetic activity of the plants.

Observed increases in soil microbial biomass could constitute an important nutrient pool, as its turnover provides a supply of available P (Bünemann et al. 2012). Rhizodeposits provide microbes with a C source, while nutrient mobilisation carried out jointly by plant roots and microbes provides good conditions for microbial growth. There is a certain competition between plants and microbes for the mobilized nutrients and a significant share of the mobilised nutrients is immobilized in microbial biomass. For the remobilization of P from microbial biomass, an active and diverse soil food web is required (Bonkowski et al. 2009). The remobilisation of immobilized P becomes even more important when we consider the great quantities of complex P forms in the supra- and macromolecular structures (McLaren et al. 2015), as these fractions could be derived to a significant extent from microbial necromass. In our experiments, soil fauna was not studied directly, although some fatty acid biomarkers suggest increases in the abundance of soil eukaryotes with cover crops (data not shown). Cover crops are generally expected to improve habitat conditions for soil fauna (Kaspar and Singer 2015), though research on specific functions of soil (micro-) fauna deserve greater attention.

8.2 How are soil microbial functions and the enzymatic availability of P_{org} pools under cover crops connected?

Field experiments of Studies #2 and #3 investigated whether stimulation of the soil microbial community is accompanied by an increase in P-cycling potential. It also explored the relationship between P_{org} substrate pools and their respective enzymes by connecting measurements of enzyme activity, enzyme-available P_{org}, and microbial abundance.

As outlined in the review section of Study #1, different enzymes, mostly produced by microbes but to some extent also by plant roots, convert soil P_{org} compounds into plant-available phosphate. To do so, phosphomonoesters are hydrolysed by phosphomonoesterases, whereas for diesters an initial dephosphorylation by a diesterase is required. Phytases represent a specialized form of monoesterases that are additionally able to initiate the cleavage of high-order inositols (Keller et al. 2012), but to date we lack generally accepted methods to measure the activities of these enzymes in soils.

We found cover crops to result in a substantial increase in potential activity of the measured phosphatases, with a larger increase in Study #3 than Study #2 (Figs 6.6 and 7.6). Acid phosphomonoesterase activity was in all cases the enzyme with the highest activity, doubling (Study #2) or even quintupling (Study #3) the activity of phosphodiesterase. The concentration of available substrate is assumed to be one of the main factors that control the production and release of phosphatases (Quiquampoix and Mousain 2005).

Our analyses revealed that the two sites contrasted in the composition of their P pools. The field in which Study #2 was conducted was dominated by inorganic P, while P_{org} was prevalent in the soil of Study #3 and the quantity of enzyme labile P_{org} was double that of Study #2 (Figs 6.3 and 7.2). Nevertheless, despite differences in P status of the two sites, added phytase was responsible for the largest release of phosphate, larger than the sum of phosphomonoesterase and phosphodiesterase. This is in line with the large proportion of recalcitrant phytate-P in soils compared to non-phytate monoesters and diesters (McLaren et al. 2020). Together, phosphomonoesters and especially phytates interact with particles and metal cations of the soil matrix (Giles et al. 2011), accumulating as a result of limited availability for enzymatic degradation. Phosphodiesters, mainly contained in nucleic acids and phospholipids, comprise the largest proportion of P_{org} in microbial biomass (Bünemann et al. 2008). However, although their mineralization is limited by the availability and ubiquity of diesterases (Lang et al. 2017; Jarosch et al. 2019), their inherent lability prevents accumulation in the soil.

An increase in enzyme-labile P_{org} pools was determined in the rhizosheaths of cover crops in Study #2 (Fig 6.3), and in 0-5 cm bulk soil in Study #3 (Fig. 7.2). These findings were supported by data on other relatively available P pools, such as microbial biomass P and

total PLFAs in both field experiments (Figs 6.4 and 7.4) and in the meta-analysis (Fig. 5.6). Interestingly, the increases in enzyme-labile P_{org} changes were driven by phosphomonoesterase- and phosphodiesterase-labile fractions in Study #2 and by phytase-labile fractions in Study #3. These findings could indicate that soil P characteristics lead to shifts in enzymatic availability under cover crops. However, more studies are necessary to identify the underlying processes. While the characterization of P_{org} pools according to their lability under added enzymes is a necessary step in understanding of soil P_{org} dynamics, open questions remain. For example, an increase in pool size could be due to several processes. Different mechanisms drive increased pool size; both increased production, and decreased degradation due to lower abundances of degraders or their activity (Guggenberger et al. 1996). Additionally, biochemical and physical processes may change the sorption dynamics of the soil and therefore the availability of specific P_{org} compounds.

Soil properties also influence the relationship between substrates and enzymes, as enzyme kinetics suggest that phosphodiesterases have a slightly higher substrate affinity than phosphomonoesterases (Acosta-Martínez and Tabatabai 2011), but the catalytic efficiency of phosphatases seems to be quite sensitive to external factors (Perucci and Scarponi 1985). Other factors, such as stabilisation and turnover times of P-cycling enzymes, as well as complexation of substrates, may be important for enzymatic turnover *in-situ* (Rao et al. 2000). While sorption, stabilization and inactivation on soil particles is described for phosphomonoesterase (Kandeler 1990) and for phytases (Giaveno et al. 2010), the consequences of these processes have been less well studied for phosphodiesterases.

Connecting the potential activities of acid phosphomonoesterase and phosphodiesterase with their respective substrates does not alone give a clear picture. In Study #3 the correlations between enzymes and their respective P_{org} substrates were consistently positive (i.e., higher enzyme production as a response to substrate availability, Fig. 7.7). In contrast, in Study #2 the enzyme-substrate-relation was more complex and was apparently influenced by the treatments and by soil depth (Fig. 6.8). These findings underscore the need for more investigation into whether these differences were caused by soil properties or by other factors, such as agricultural management.

8.3 Agricultural management and plant-soil-microbe interactions

Management decisions greatly impact the effects of agricultural practices on plant-soil-microbe interactions. In the case of cover crops, one important factor is the choice of cover crop species, as outlined in Study #1, but secondary management decisions such as fertilization, tillage, termination method (mechanical or chemical, e.g., with glyphosate), or irrigation (Romdhane et al. 2019; Kim et al. 2020b; Ortega et al. 2021) are also important. Our findings show that agricultural management as well as crop species choice can be used to optimize the cover crop-derived P benefit. To determine the implications for soil ecology, a convenient perspective is to identify how these management decisions affect the quantity, quality, and placement of substrates, along with other physicochemical modifications of the soil that affect the soil biota. These factors all impact soil ecology more broadly.

The *plant biomass pathway*, more important in settings with a high biomass-producing cover crop and in soils with low to moderate sorption capacity, is likely the pathway that is most directly affected by agricultural management. To improve the efficiency of this pathway, decomposition dynamics of cover crop litter and timing of the most important P uptake phases of the main crops need to be synchronized (Bünemann et al. 2004). The greatest potential for optimizing decomposition dynamics resides in both the choice of cover crop species, which controls litter C:N:P ratios, and management decisions such as termination method or residue management (i.e., tillage vs surface mulch layer), although soil chemistry and climate remain controlling factors. Study #1 revealed that cover crops with high C:P ratios, such as Poacea, frequently failed to increase yields or P content of the main crop (Fig. 5.4). Cover crop mixtures have potential, but single-species cover crops can also produce impressive amounts of biomass, as seen in the case of mustard, presented in Study #2 (Fig 6.2).

The large number of potential cover crop species and crop rotations represents a challenge for the assessment of cover crop-related effects. An especially promising approach to deal with the plethora of possible combinations of cover crop mixtures and main crops is the use of plant functional traits to characterise plant species (Honvault et al. 2020, 2021). In an exhaustive study conducted by Wendling et al. (2016), cover crop species were grouped according to nutrient uptake rates and plant properties. Shoot and root traits

rather than taxonomy were identified as the main drivers of this clustering. However, biochemical and microbial root P-acquisition strategies were not assessed, which might well be relevant in systems with limited P availability.

Detectable cover crop effects on biological and chemical properties of the soil occurred mainly in the compartments directly in contact with the plants, namely around plant roots in the rhizosheaths (Study #2), and at the soil surface, the detritusphere (Study #3). In the bulk soil surrounding the rhizosheaths and in the deeper soil layers, cover crop effects were much lower. The observed effects on soil microbes due to differences in soil compartments highlight the need to estimate and take into account the extensions of the rhizosheaths. It appears that cover crops mainly affect the hotspots where soil microbial abundance and activity is already at a high level. Given this, the effects of soil heterogeneity at different scales must be determined in order to improve our ability to predict soil ecological processes at the field scale (Regan et al. 2014).

Tillage and residue placement effects on nutrient release can be important, but seem not to be straightforward. Incorporation of residues may increase C mineralization rates, with water availability possibly one of the main limiting factors for microbial decomposition of surface-placed litter (McCourty et al. 2018), but this is probably of minor importance in a central European winter. Cover crops increased soybean residue decomposition in a no-till system in Brazil by 6-8 %, controlled by microbial biomass and activity, as well as soil moisture, but did not affect P release (Varela et al. 2014). These findings indicate that different drivers are responsible for C and P mineralization. The study also questioned the generality of a critical threshold for P immobilization, as P release occurred despite a relatively high C:P, which could be related to differences in climate and soil. To date, there is not enough data to draw solid conclusions about potential tillage effects on P release of plant residues (Damon et al. 2014).

9 Conclusions and Perspectives

The findings presented here have broad implications for our understanding of soil P_{org} cycling with cover crops and beyond. We demonstrated that plants can shift P toward pools of increased availability in active soil compartments and confirmed the important role of microbes in these processes. From a basic research perspective, the characterisation of P_{org} according to its availability to enzymes adds knowledge to our understanding of terrestrial P cycling. From an applied perspective, our results can be translated into relevant messages for farmers. Besides the potential to manage nutrients with cover crops, the conceptual framework of cover crop-derived P benefit offers an opportunity to consider both pre-crop effects and the rotational value of plant species as elements of a crop rotation (Marques et al. 2020). Additionally, this concept can be taken into account when building similar frameworks for other plant nutrients, such as silicon (de Tombeur et al. 2021).

Given the scarcity of P, some have expressed the need to transition toward a circular P economy (Withers et al. 2018). Cover crops have the potential to convert the legacy P of past fertilizer applications into a usable resource (Menezes-Blackburn et al. 2018). To actually close the cycle of our limited P resources, it will be increasingly necessary to use recovered P from waste streams, which will be utilized frequently in organic forms. Therefore, understanding how plants access organic P forms is of vital interest for future plant production systems (Stutter et al. 2012). Integration of crop P acquisition strategies and root traits (Honvault et al. 2020), matching organic fertilisers (Nobile et al. 2019), mineralisation dynamics (Damon et al. 2014), effects on soil P sorption (Iyamuremye et al. 1996), and spatial distribution of released P (Christel et al. 2016), together have great potential for more strategic nutrient management (Drinkwater and Snapp 2007).

Besides its application in agricultural settings, the framework presented here can possibly be adapted to plant succession in natural ecosystems. While agroecosystems are clearly different from natural ecosystems, in particular regarding their reduced microbial diversity and complete succession in annual cropping systems, the described pathways of P benefit could still be important, also regarding the success of invasive exotic species (Chang and Turner 2019). The effect of a preceeding plant on P availability would likely be more important for P recycling ecosystems in soils with low mineral P content (Lang et al. 2017).

From a methodological point of view, characterisation of the P_{org} pools according to their availability for enzymes, as performed in this thesis, could be extended to other elements that exist in a significant share as organic forms, such as C and N. The recent developments of methods to study soil P_{org} (e.g., ^{31}P NMR) permit greater insights into the chemical nature of these pools (McLaren et al. 2020). However, it has become increasingly evident that, in order to decipher the biogeochemical cycling of P in soils, we need to improve our understanding of microbial ecology, in particular related to the accessibility of P_{org}. My own perspective is that this somehow mirrors current discussions in the field of humics research; that soil organic matter is a heterogeneous continuum of components in decomposition rather than an assemblage of macromolecules (Baveye and Wander 2019). At the same time, it highlights the potential for characterising soil nutrient pools according to their availability to enzymes, intrinsically connected to degradability. It is plausible that, analogous to SOM, a significant amount of unidentified P_{org} in soils is associated with microbial necromass, which would explain the association of microbial biomass and turnover with P_{org}.

Many opportunities remain to conduct research on microbial functions and their roles in the regulation of ecological processes. A more comprehensive strategy for understanding biological functions needs to include improvement of our analytical methods (Alteio et al. 2021). This is especially evident for soil fungi, as the current molecular tools are heavily skewed towards bacteria, to the detriment of soil fungi. The role of soil biodiversity for the provision of ecosystem functions is increasingly acknowledged (Crotty et al. 2022), and management techniques such as cover crops are proposed for optimizing these functions (Vazquez et al. 2021). A study by Kim et al. (2020a) suggests that cover crops increase the abundance and activity of microbes more than the diversity of the soil microbial community, but further research is necessary. The development of novel omics approaches will improve our capacity to assess the functions of the soil microbiome (Bertola et al. 2021), contributions of the different microbial groups, and, hopefully, opportunities for (agricultural) management to optimize the provided functions. The structural inclusion of biological parameters into soil quality assessments has great potential both to improve our

knowledge of the underlying biological processes, and to increase our ability to predict the outcome of management changes (Bünemann et al. 2018).

The inclusion of cover crops into the crop rotation is an important step in the route to more sustainable agriculture, as this inclusion might constitute an entry point for farmers to build their farming activity on the services provided by a healthy agroecosystem rather than by omitting (or even working against) these functions. As stated by José Graziano da Silva, Director General of the United Nations Food and Agriculture Organization (FAO) at the opening of the 2nd Agroecology Symposium 2018 in Rome, Italy, there is urgency "*to get out of the trap of conventional, high-resource input systems with increasing productivity at any social and ecological costs, still not leading out of hunger for over 800 million people*" (Flury 2018). Deeper and more fundamental structural changes are needed, including extension of the concept of agroecology from field management to factors such as land tenure and control over research and technologies (Wezel et al. 2020).

Although agricultural production systems that rely on nature-based soil improving cropping systems and the ecosystem services they provide constitute probably a more sustainable and resilient option than reliance on technological inputs (e.g., agrochemicals, robots, …), there are two main obstacles that need to be addressed.

First, the dependence on favourable climatic conditions for yield stability poses a looming threat for future food security (Knapp and van der Heijden 2018). It must be acknowledged that, although cover crops represent a measure of adaption to some aspects of climate change, e.g., by increasing infiltration or reducing erosion caused by heavy rainfalls, cover cropping systems tailored to specific socio-environmental local conditions are still to be developed (Kaye and Quemada 2017).

The second obstacle to greater cover crop adoption is related to the direct economic returns by switching to more sustainable agricultural practices (Bergtold et al. 2019). In this set of studies, we found clear indications that the shifting of soil P_{org} toward pools of greater availability constitutes an important mechanism of cover crop effects on nutrient cycling. But to what degree does this improved access to P benefit the following main crop? While the meta-analysis showed a positive trend, albeit with considerable variability, our own field experiments did not show an improvement in soybean P nutrition. This shows yet again the

complexity of on-farm experiments, since in Study #3, conducted on a more conventionally managed field, the cover crop-related shifts in soil biological properties persisted over time, while in Study #2 these effects were more transient. Site conditions, climate, and agricultural management are important factors that influence the outcome of cover cropping (García-González et al. 2018). Notwistanding these factors, cover crop management is key for successful conservation agriculture (Mirsky et al. 2012), but the lack of this type of management experience results in higher risks and the potential to make mistakes with these knowledge-intensive systems (Zikeli and Gruber 2017). In practice, farmers who experiment with no-till techniques are increasingly adopting a modified approach coined as *occasional tillage* (Peixoto et al. 2020). This approach consists of very shallow non-inversion tillage (chisel plow) as an emergency measure in certain situations, e.g., when the cover crop emergence fails and/or high weed pressure appears. In combination with *occasional tillage*, the weed-suppressing abilities of cover crops offer the potential to reduce herbicide use substantially (Zikeli and Gruber 2017), which is currently a major demand of the public.

To overcome these barriers to adoption of soil improving cropping systems, transdisciplinary, large-scale experiments, in full cooperation with practitioners, are required to develop working examples of best practices (Junge et al. 2020). External benefits for society, such as soil, water, and biodiversity protection, as well as increased C sequestration, which are currently not reflected in the market value of the crops, also need to be taken into account (Dendoncker et al. 2018). Inclusion of cover crops is also based on different rationales for different cropping systems, and will result in varying benefits. For example, a cover crop mulch layer for vegetable production can result in similar economic returns as a conventional system and provide additional, non-monetary benefits (Creamer et al. 1996).

Soil erosion is currently, and will continue to be in the near future, the greatest threat to agriculture, since it is responsible for impressive nutrient losses (Borrelli et al. 2020). One of the principal benefits of broad adoption of cover cropping may well be related to erosion reduction (Alewell et al. 2020). Cover crops can tackle multiple problems while simultaneously providing as side effects positive outcomes on soil biological processes and

P_{org} cycling. This is fortunate, as some administrations are still rather unethusiastic concerning the consideration of other P forms than phosphate in their national frameworks for fertilizer recommendations (Tóth et al. 2014).

In conclusion, our findings suggest that cover crops can increase access to P for main crops via different pathways. Our field experiments confirmed the potential cycling of P through the cover crop biomass and we were able to relate observed increases in the availability of P_{org} to microbial abundance and activity, with soil fungi playing an important role. The selection of cover crop species and management decisions can be optimized and adapted to local conditions. All in all, this new knowledge about soil phosphorus cycling in agroecosystems will help us to improve management of the limited P resource for a more sustainable agriculture.

10 References

Abdollahi L, Munkholm LJ (2014) Tillage system and cover crop effects on soil quality: I. chemical, mechanical, and biological properties. Soil Sci Soc Am J 78:262–270. https://doi.org/10.2136/sssaj2013.07.0301

Acosta-Martínez V, Tabatabai MA (2011) Phosphorus cycle enzymes. In: Dick RP (ed) Methods of Soil Enzymology. Soil Science Society of America, Madison, USA, pp 161–183

Aerts R, Bakker C, De Caluwe H (1992) Root turnover as determinant of the cycling of C, N, and P in a dry heathland ecosystem. Biogeochemistry 15:175–190. https://doi.org/10.1007/BF00002935

Alewell C, Ringeval B, Ballabio C, et al (2020) Global phosphorus shortage will be aggravated by soil erosion. Nat Commun 11:4546. https://doi.org/10.1038/s41467-020-18326-7

Almeida DS, Rosolem CA (2016) Ruzigrass grown in rotation with soybean increases soil labile phosphorus. Agron J 108:2444–2452. https://doi.org/10.2134/agronj2015.0478

Alteio LV, Séneca J, Canarini A, et al (2021) A critical perspective on interpreting amplicon sequencing data in soil ecological research. Soil Biol Biochem 160:108357. https://doi.org/10.1016/j.soilbio.2021.108357

Altieri MA (2002) Agroecology: the science of natural resource management for poor farmers in marginal environments. Agric Ecosyst Environ 93:1–24. https://doi.org/10.1016/S0167-8809(02)00085-3

Altieri MA (2018) Agroecology: the science of sustainable agriculture, 2nd edn. CRC Press, Boca Raton

Alvarez R, Steinbach HS, De Paepe JL (2017) Cover crop effects on soils and subsequent crops in the pampas: A meta-analysis. Soil Tillage Res 170:53–65. https://doi.org/10.1016/j.still.2017.03.005

Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol Biochem 10:215–221. https://doi.org/10.1016/0038-0717(78)90099-8

Annaheim KE, Rufener CB, Frossard E, Bünemann EK (2013) Hydrolysis of organic phosphorus in soil water suspensions after addition of phosphatase enzymes. Biol Fertil Soils 49:1203–1213. https://doi.org/10.1007/s00374-013-0819-1

Anugroho F, Kitou M, Nagumo F, et al (2009) Growth, nitrogen fixation, and nutrient uptake of hairy vetch as a cover crop in a subtropical region. Weed Biol Manag 9:63–71. https://doi.org/10.1111/j.1445-6664.2008.00319.x

Arcand MM, Lynch DH, Voroney RP, van Straaten P (2010) Residues from a buckwheat (*Fagopyrum esculentum*) green manure crop grown with phosphate rock influence bioavailability of soil phosphorus. Can J Soil Sci 90:257–266. https://doi.org/10.4141/CJSS09023

Aristarán M, Tigas M, Jeremy B. Merrill, Jason D (2017) Tabula: Extract tables from pdfs. Version 1.1.1URL http://tabula.technology/

Aronsson H, Hansen EM, Thomsen IK, et al (2016) The ability of cover crops to reduce nitrogen and phosphorus losses from arable land in southern Scandinavia and Finland. J Soil Water Conserv 71:41–55. https://doi.org/10.2489/jswc.71.1.41

Austin EE, Wickings K, McDaniel MD, et al (2017) Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. GCB Bioenergy 9:1252–1263. https://doi.org/10.1111/gcbb.12428

Azeem M, Riaz A, Chaudhary AN, et al (2015) Microbial phytase activity and their role in organic P mineralization. Arch Agron Soil Sci 61:751–766. https://doi.org/10.1080/03650340.2014.963796

Bååth E (2003) The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. Microb Ecol 45:373–383. https://doi.org/10.1007/s00248-003-2002-y

Bachmann S, Eichler-Löbermann B (2010) Soil phosphorus pools as affected by application of poultry litter ash in combination with catch crop cultivation. Commun Soil Sci Plant Anal 41:1098–1111. https://doi.org/10.1080/00103621003687182

Bagayoko M, Buerkert A, Lung G, et al (2000) Cereal/legume rotation effects on cereal growth in Sudano-Sahelian West Africa: soil mineral nitrogen, mycorrhizae and nematodes. Plant Soil 218:103–116. https://doi.org/10.1023/A:1014957605852

Bah AR, Zaharah AR, Hussin A (2006) Phosphorus uptake from green manures and phosphate fertilizers applied in an acid tropical soil. Commun Soil Sci Plant Anal 37:2077–2093. https://doi.org/10.1080/00103620600770433

Balota EL, Calegari A, Nakatani AS, Coyne MS (2014) Benefits of winter cover crops and no-tillage for microbial parameters in a Brazilian Oxisol: A long-term study. Agric Ecosyst Environ 197:31–40. https://doi.org/10.1016/j.agee.2014.07.010

Barber SA (1995) Soil nutrient bioavailability: A mechanistic approach. John Wiley & Sons, New York

Bardgett RD, Hobbs PJ, Frostegard A (1996) Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. Biol Fertil Soils 22:261–264. https://doi.org/10.1007/BF00382522

Barrett-Lennard EG, Dracup M, Greenway H (1993) Role of extracellular phosphatases in the phosphorus-nutrition of clover. J Exp Bot 44:1595–1600. https://doi.org/10.1093/jxb/44.10.1595

Bashan Y, Kamnev AA, de-Bashan LE (2013) Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: a proposal for an alternative procedure. Biol Fertil Soils 49:465–479. https://doi.org/10.1007/s00374-012-0737-7

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Baveye PC, Wander M (2019) The (bio)chemistry of soil humus and humic substances: why is the “New View” still considered novel after more than 80 years? Front Environ Sci 7:27. https://doi.org/10.3389/fenvs.2019.00027

Beck T, Joergensen RG, Kandeler E, et al (1997) An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biol Biochem 29:1023–1032. https://doi.org/10.1016/S0038-0717(97)00030-8

Belinque H, Pucheu N, Kerber N, Rubio G (2015) Utilization of organic phosphorus sources by oilseed rape, sunflower, and soybean. J Plant Nutr Soil Sci 178:339–344. https://doi.org/10.1002/jpln.201400301

Bending GD, Aspray TJ, Whipps JM (2006) Significance of microbial interactions in the mycorrhizosphere. Adv Appl Microbiol 60:97–132. https://doi.org/10.1016/S0065-2164(06)60004-X

Benitez M-S, Taheri WI, Lehman RM (2016) Selection of fungi by candidate cover crops. Appl Soil Ecol 103:72–82. https://doi.org/10.1016/j.apsoil.2016.03.016

Bergkemper F, Kublik S, Lang F, et al (2016) Novel oligonucleotide primers reveal a high diversity of microbes which drive phosphorous turnover in soil. J Microbiol Methods 125:91–97. https://doi.org/10.1016/j.mimet.2016.04.011

Bergström L, Kirchmann H, Djodjic F, et al (2015) Turnover and losses of phosphorus in swedish agricultural soils: long-term changes, leaching trends, and mitigation measures. J Environ Qual 44:512–523. https://doi.org/10.2134/jeq2014.04.0165

Bergtold JS, Ramsey S, Maddy L, Williams JR (2019) A review of economic considerations for cover crops as a conservation practice. Renew Agric Food Syst 34:62–76. https://doi.org/10.1017/S1742170517000278

Bernoux M, Cerri CC, Cerri CEP, et al (2006) Cropping systems, carbon sequestration and erosion in Brazil, a review. Agron Sustain Dev 26:1–8. https://doi.org/10.1051/agro:2005055

Bertola M, Ferrarini A, Visioli G (2021) Improvement of soil microbial diversity through sustainable agricultural practices and its evaluation by -omics approaches: a perspective for the environment, food quality and human safety. Microorganisms 9:1400. https://doi.org/10.3390/microorganisms9071400

Bier RL, Bernhardt ES, Boot CM, et al (2015) Linking microbial community structure and microbial processes: an empirical and conceptual overview. FEMS Microbiol Ecol 91:1–11. https://doi.org/10.1093/femsec/fiv113

Bittman S, Kowalenko CG, Hunt DE, et al (2006) Starter phosphorus and broadcast nutrients on corn with contrasting colonization by Mycorrhizae. Agron J 98:394–401. https://doi.org/10.2134/agronj2005.0093

Blanchart E, Villenave C, Viallatoux A, et al (2006) Long-term effect of a legume cover crop (*Mucuna pruriens* var. *utilis*) on the communities of soil macrofauna and nematofauna, under maize cultivation, in southern Benin. Eur J Soil Biol 42:S136–S144. https://doi.org/10.1016/j.ejsobi.2006.07.018

Blanco-Canqui H, Shaver TM, Lindquist JL, et al (2015) Cover crops and ecosystem services: insights from studies in temperate soils. Agron J 107:2449–2474. https://doi.org/10.2134/agronj15.0086

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917. https://doi.org/10.1139/o59-099

Boeddinghaus RS, Marhan S, Berner D, et al (2019) Plant functional trait shifts explain concurrent changes in the structure and function of grassland soil microbial communities. J Ecol 107:2197–2210. https://doi.org/10.1111/1365-2745.13182

Boglaienko D, Soti P, Shetty KG, Jayachandran K (2014) Buckwheat as a cover crop in Florida: Mycorrhizal status and soil analysis. Agroecol Sustain Food Syst 38:1033–1046. https://doi.org/10.1080/21683565.2014.906016

Bommarco R, Kleijn D, Potts SG (2013) Ecological intensification: harnessing ecosystem services for food security. Trends Ecol Evol 28:230–238. https://doi.org/10.1016/j.tree.2012.10.012

Bonkowski M, Villenave C, Griffiths B (2009) Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots. Plant Soil 321:213–233. https://doi.org/10.1007/s11104-009-0013-2

Borie F, Zunino H (1983) Organic matter-phosphorus associations as a sink in P-fixation processes in allophanic soils of Chile. Soil Biol Biochem 15:599–603

Borrelli P, Robinson DA, Panagos P, et al (2020) Land use and climate change impacts on global soil erosion by water (2015-2070). Proc Natl Acad Sci 117:21994–22001. https://doi.org/10.1073/pnas.2001403117

Boselli R, Fiorini A, Santelli S, et al (2020) Cover crops during transition to no-till maintain yield and enhance soil fertility in intensive agro-ecosystems. Field Crops Res 255:107871. https://doi.org/10.1016/j.fcr.2020.107871

Boswell EP, Koide RT, Shumway DL, Addy HD (1998) Winter wheat cover cropping, VA mycorrhizal fungi and maize growth and yield. Agric Ecosyst Environ 67:55–65. https://doi.org/10.1016/S0167-8809(97)00094-7

Bowles TM, Jackson LE, Loeher M, Cavagnaro TR (2017) Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. J Appl Ecol 54:1785–1793. https://doi.org/10.1111/1365-2664.12815

Bowman RA (1989) A sequential extraction procedure with concentrated sulfuric acid and dilute base for soil organic phosphorus. Soil Sci Soc Am J 53:362–366. https://doi.org/10.2136/sssaj1989.03615995005300020008x

Bowman RA, Moir JO (1993) Basic EDTA as an extractant for soil organic phosphorus. Soil Sci Soc Am J 57:1516–1518. https://doi.org/10.2136/sssaj1993.03615995005700060020x

Bray RH, Kurtz LT (1945) Determination of total, organic, and available forms of phosphorus in soils. Soil Sci 59:39–46

Brennan EB (2017) Can we grow organic or conventional vegetables sustainably without cover crops? HortTechnology 27:151–161. https://doi.org/10.21273/HORTTECH03358-16

Brennan EB, Acosta-Martinez V (2017) Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production. Soil Biol Biochem 109:188–204. https://doi.org/10.1016/j.soilbio.2017.01.014

Brennan EB, Boyd NS, Smith RF, Foster P (2009) Seeding rate and planting arrangement effects on growth and weed suppression of a legume-oat cover crop for organic vegetable systems. Agron J 101:979–988. https://doi.org/10.2134/agronj2008.0194x

Brookes PC, Powlson DS, Jenkinson DS (1982) Measurement of microbial biomass phosphorus in soil. Soil Biol Biochem 14:319–329. https://doi.org/10.1016/0038-0717(82)90001-3

Brophy LS, Heichel GH, Russelle MP (1987) Nitrogen transfer from forage legumes to grass in a systematic planting design. Crop Sci 27:753–758

Büchi L, Wendling M, Amossé C, et al (2018) Importance of cover crops in alleviating negative effects of reduced soil tillage and promoting soil fertility in a winter wheat cropping system. Agric Ecosyst Environ 256:92–104. https://doi.org/10.1016/j.agee.2018.01.005

Bünemann E, Oberson A, Frossard E (eds) (2011) Phosphorus in action: Biological processes in soil phosphorus cycling. Springer, Berlin, Heidelberg

Bünemann EK (2008) Enzyme additions as a tool to assess the potential bioavailability of organically bound nutrients. Soil Biol Biochem 40:2116–2129. https://doi.org/10.1016/j.soilbio.2008.03.001

Bünemann EK (2015) Assessment of gross and net mineralization rates of soil organic phosphorus–A review. Soil Biol Biochem 89:82–98

Bünemann EK, Bongiorno G, Bai Z, et al (2018) Soil quality – A critical review. Soil Biol Biochem 120:105–125. https://doi.org/10.1016/j.soilbio.2018.01.030

Bünemann EK, Bossio DA, Smithson PC, et al (2004) Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization. Soil Biol Biochem 36:889–901. https://doi.org/10.1016/j.soilbio.2004.02.002

Bünemann EK, Marschner P, McNeill AM, McLaughlin MJ (2007) Measuring rates of gross and net mineralisation of organic phosphorus in soils. Soil Biol Biochem 39:900–913. https://doi.org/10.1016/j.soilbio.2006.10.009

Bünemann EK, Oberson A, Liebisch F, et al (2012) Rapid microbial phosphorus immobilization dominates gross phosphorus fluxes in a grassland soil with low inorganic phosphorus availability. Soil Biol Biochem 51:84–95. https://doi.org/10.1016/j.soilbio.2012.04.012

Bünemann EK, Smernik RJ, Marschner P, McNeill AM (2008) Microbial synthesis of organic and condensed forms of phosphorus in acid and calcareous soils. Soil Biol Biochem 40:932–946. https://doi.org/10.1016/j.soilbio.2007.11.012

Burak E, Quinton JN, Dodd IC (2021) Root hairs are the most important root trait for rhizosheath formation of barley (*Hordeum vulgare*), maize (*Zea mays*) and *Lotus japonicus* (Gifu). Ann Bot 128:45–57. https://doi.org/10.1093/aob/mcab029

Burns RG (1982) Enzyme activity in soil: Location and a possible role in microbial ecology. Soil Biol Biochem 14:423–427. https://doi.org/10.1016/0038-0717(82)90099-2

Burns RG, Dick RP (eds) (2002) Enzymes in the environment: activity, ecology, and applications, 1st edn. CRC Press, New York

Buyer JS, Teasdale JR, Roberts DP, et al (2010) Factors affecting soil microbial community structure in tomato cropping systems. Soil Biol Biochem 42:831–841. https://doi.org/10.1016/j.soilbio.2010.01.020

Calegari A, Tiecher T, Hargrove WL, et al (2013) Long-term effect of different soil management systems and winter crops on soil acidity and vertical distribution of

nutrients in a Brazilian Oxisol. Soil Tillage Res 133:32–39. https://doi.org/10.1016/j.still.2013.05.009

Calonego JC, Rosolem CA (2010) Soybean root growth and yield in rotation with cover crops under chiseling and no-till. Eur J Agron 33:242–249. https://doi.org/10.1016/j.eja.2010.06.002

Campbell BM, Beare DJ, Bennett EM, et al (2017) Agriculture production as a major driver of the Earth system exceeding planetary boundaries. Ecol Soc 22:11

Cardoso DP, Silva MLN, De C, et al (2013) Potential of cover plant species in reconditioning of physical and chemical properties of soil. Rev Bras Cienc Agrar 8:375–382. https://doi.org/10.5039/agraria.v8i3a2421

Carpenter SR, Bennett EM (2011) Reconsideration of the planetary boundary for phosphorus. Environ Res Lett 6:1–12. https://doi.org/10.1088/1748-9326/6/1/014009

Carsky RJ, Oyewole B, Tian G (2001) Effect of phosphorus application in legume cover crop rotation on subsequent maize in the savanna zone of West Africa. Nutr Cycl Agroecosystems 59:151–159

Castillo-Caamal JB, Caamal-Maldonado JA (2011) Efecto de la fecha de siembra del frijol terciopelo (*Mucuna* sp.) como cultivo de cobertera en el rendimiento de maíz. Trop Subtrop Agroecosystems 14:101–108

Cavigelli MA, Thien SJ (2003) Phosphorus bioavailability following incorporation of green manure crops. Soil Sci Soc Am J 67:1186–1194

Ceci A, Pinzari F, Russo F, et al (2018) Saprotrophic soil fungi to improve phosphorus solubilisation and release: In vitro abilities of several species. Ambio 47:30–40. https://doi.org/10.1007/s13280-017-0972-0

Ceulemans T, Stevens CJ, Duchateau L, et al (2014) Soil phosphorus constrains biodiversity across European grasslands. Glob Change Biol 20:3814–3822. https://doi.org/10.1111/gcb.12650

Chang CC, Turner BL (2019) Ecological succession in a changing world. J Ecol 107:503–509. https://doi.org/10.1111/1365-2745.13132

Cherr CM, Scholberg JMS, McSorley R (2006) Green manure approaches to crop production. Agron J 98:302–319. https://doi.org/10.2134/agronj2005.0035

Chien SH, Prochnow LI, Tu S, Snyder CS (2011) Agronomic and environmental aspects of phosphate fertilizers varying in source and solubility: an update review. Nutr Cycl Agroecosystems 89:229–255. https://doi.org/10.1007/s10705-010-9390-4

Christel W, Zhu K, Hoefer C, et al (2016) Spatiotemporal dynamics of phosphorus release, oxygen consumption and greenhouse gas emissions after localised soil amendment with organic fertilisers. Sci Total Environ 554–555:119–129. https://doi.org/10.1016/j.scitotenv.2016.02.152

Clark A (2008) Managing cover crops profitably, 3rd edn. DIANE Publishing, Darby, PA

Clarkson DT (1985) Factors affecting mineral nutrient acquisition by plants. Annu Rev Plant Physiol 36:77–115

Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? Biogeochemistry 85:235–252. https://doi.org/10.1007/s10533-007-9132-0

Clocchiatti A, Hannula SE, van den Berg M, et al (2020) The hidden potential of saprotrophic fungi in arable soil: Patterns of short-term stimulation by organic amendments. Appl Soil Ecol 147:103434. https://doi.org/10.1016/j.apsoil.2019.103434

Cole CV, Elliott ET, Hunt HW, Coleman DC (1977) Trophic interactions in soils as they affect energy and nutrient dynamics. V. Phosphorus transformations. Microb Ecol 4:381–387

Colwell JD (1963) The estimation of the phosphorus fertilizer requirements of wheat in southern New South Wales by soil analysis. Aust J Exp Agric 3:190–197. https://doi.org/10.1071/ea9630190

Condron LM, Frossard E, Tiessen H, et al (1990) Chemical nature of organic phosphorus in cultivated and uncultivated soils under different environmental conditions. J Soil Sci 41:41–50. https://doi.org/10.1111/j.1365-2389.1990.tb00043.x

Condron LM, Turner BL, Cade-Menun BJ (2005) Chemistry and dynamics of soil organic phosphorus. In: Sims JT, Sharpley AN (eds) Phosphorus: agriculture and the environment. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, USA

Cordell D, White S (2014) Life’s bottleneck: Sustaining the world’s phosphorus for a food secure future. Annu Rev Environ Resour 39:161–188. https://doi.org/10.1146/annurev-environ-010213-113300

Costa SEVGA, Souza ED, Anghinoni I, et al (2014) Impact of an integrated no-till crop–livestock system on phosphorus distribution, availability and stock. Agric Ecosyst Environ 190:43–51. https://doi.org/10.1016/j.agee.2013.12.001

Cozzolino V, Di Meo V, Piccolo A (2013) Impact of arbuscular mycorrhizal fungi applications on maize production and soil phosphorus availability. J Geochem Explor 129:40–44. https://doi.org/10.1016/j.gexplo.2013.02.006

Creamer NG, Dabney SM (2002) Killing cover crops mechanically: Review of recent literature and assessment of new research results. Am J Altern Agric 17:32–40

Cross AF, Schlesinger WH (1995) A literature review and evaluation of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. Geoderma 64:197–214. https://doi.org/10.1016/0016-7061(94)00023-4

Crossland M, Fradgley N, Creissen H, et al (2015) An online toolbox for cover crops and living mulches. In: Aspects of Applied Biology. Association of Applied Biologists, Warwick, UK, pp 1–6

Crotty F, Hannula E, Hallama M, Kandeler E (2022) Can soil improving cropping systems reduce the loss of soil biodiversity within agricultural soils? In the book. In: Reyes-Sánchez LB, Horn R, Constantini EAC (eds) Sustainable soil management as a key to preserving soil biodiversity and stopping its degradation, IUSS Book in edition process

Cu STT, Hutson J, Schuller KA (2005) Mixed culture of wheat (*Triticum aestivum* L.) with white lupin (*Lupinus albus* L.) improves the growth and phosphorus nutrition of the wheat. Plant Soil 272:143–151. https://doi.org/10.1007/s11104-004-4336-8

Cui H, Zhou Y, Gu Z, et al (2015) The combined effects of cover crops and symbiotic microbes on phosphatase gene and organic phosphorus hydrolysis in subtropical orchard soils. Soil Biol Biochem 82:119–126. https://doi.org/10.1016/j.soilbio.2015.01.003

Dabney SM, Delgado JA, Reeves DW (2001) Using winter cover crops to improve soil and water quality. Commun Soil Sci Plant Anal 32:1221–1250. https://doi.org/10.1081/CSS-100104110

Dahlin AS, Stenberg M (2009) Cutting regime affects the amount and allocation of symbiotically fixed N in green manure leys. Plant Soil 331:401–412. https://doi.org/10.1007/s11104-009-0261-1

Damon PM, Bowden B, Rose T, Rengel Z (2014) Crop residue contributions to phosphorus pools in agricultural soils: A review. Soil Biol Biochem 74:127–137. https://doi.org/10.1016/j.soilbio.2014.03.003

Damour G, Dorel M, Quoc HT, et al (2014) A trait-based characterization of cover plants to assess their potential to provide a set of ecological services in banana cropping systems. Eur J Agron 52:218–228. https://doi.org/10.1016/j.eja.2013.09.004

Dao TH, Schomberg HH, Cavigelli MA (2015) Tillage and rotational effects on exchangeable and enzyme-labile phosphorus forms in conventional and organic cropping systems. Nutr Cycl Agroecosystems 101:153–165. https://doi.org/10.1007/s10705-014-9665-2

de Tombeur F, Roux P, Cornelis J-T (2021) Silicon dynamics through the lens of soil-plant-animal interactions: perspectives for agricultural practices. Plant Soil 467:1–28. https://doi.org/10.1007/s11104-021-05076-8

Dechassa N, Schenk MK, Claassen N, Steingrobe B (2003) Phosphorus efficiency of cabbage (*Brassica oleraceae* L. var. *capitata*), carrot (*Daucus carota* L.), and potato (*Solanum tuberosum* L.). Plant Soil 250:215–224

Deguchi S, Shimazaki Y, Uozumi S, et al (2007) White clover living mulch increases the yield of silage corn via arbuscular mycorrhizal fungus colonization. Plant Soil 291:291–299. https://doi.org/10.1007/s11104-007-9194-8

Dendoncker N, Boeraeve F, Crouzat E, et al (2018) How can integrated valuation of ecosystem services help understanding and steering agroecological transitions? Ecol Soc 23:. https://doi.org/10.5751/ES-09843-230112

Deubel A, Gransee A, Merbach W (2000) Transformation of organic rhizodepositions by rhizosphere bacteria and its influence on the availability of tertiary calcium phosphate. J Plant Nutr Soil Sci 163:387–392. https://doi.org/10.1002/1522-2624(200008)163:4<387::AID-JPLN387>3.0.CO;2-K

Deubel A, Merbach W (2005) Influence of microorganisms on phosphorus bioavailability in soils. In: Varma A, Buscot F (eds) Microorganisms in Soils: Roles in Genesis and Functions. Springer, Berlin, Heidelberg, pp 177–191

Devi M, King S, Nivas D, Chandru S (2013) Enhancement of soil fertility through agro inputs on response to cover crop of *crotalaria juncea* L. In: Velu RK (ed) Microbiological Research in Agroecosystem Management, Springer. New Delhi, pp 175–186

Dijkstra FA, Carrillo Y, Pendall E, Morgan JA (2013) Rhizosphere priming: a nutrient perspective. Front Microbiol 4:1–8. https://doi.org/10.3389/fmicb.2013.00216

Dinesh R, Suryanarayana MA, Ghoshal Chaudhuri S, Sheeja TE (2004) Long-term influence of leguminous cover crops on the biochemical properties of a sandy clay loam Fluventic Sulfaquent in a humid tropical region of India. Soil Tillage Res 77:69–77. https://doi.org/10.1016/j.still.2003.11.001

Dinkelaker B, Römheld V, Marschner H (1989) Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell Environ 12:285–292

Ditterich F, Poll C, Pagel H, et al (2013) Succession of bacterial and fungal 4-chloro-2-methylphenoxyacetic acid degraders at the soil–litter interface. FEMS Microbiol Ecol 86:85–100. https://doi.org/10.1111/1574-6941.12131

Ditterich F, Poll C, Pronk GJ, et al (2016) Succession of soil microbial communities and enzyme activities in artificial soils. Pedobiologia 59:93–104. https://doi.org/10.1016/j.pedobi.2016.03.002

Dorado J, Zancada M-C, Almendros G, López-Fando C (2003) Changes in soil properties and humic substances after long-term amendments with manure and crop residues in dryland farming systems. J Plant Nutr Soil Sci 166:31–38. https://doi.org/10.1002/jpln.200390009

Dorn B, Stadler M, van der Heijden M, Streit B (2013) Regulation of cover crops and weeds using a roll-chopper for herbicide reduction in no-tillage winter wheat. Soil Tillage Res 134:121–132. https://doi.org/10.1016/j.still.2013.07.014

Douds DD, Nagahashi G, Hepperly PR (2010) On-farm production of inoculum of indigenous arbuscular mycorrhizal fungi and assessment of diluents of compost for inoculum production☆ Bioresour Technol 101:2326–2330. https://doi.org/10.1016/j.biortech.2009.11.071

Douds DD, Nagahashi G, Wilson DO, Moyer J (2011) Monitoring the decline in AM fungus populations and efficacy during a long term bare fallow. Plant Soil 342:319–326. https://doi.org/10.1007/s11104-010-0697-3

Dreymann S, Loges R, Taube F (2005) Schnittgutabfuhr oder Gründüngung? Auswirkung der Kleegras-Nutzung auf Nitrat im Sickerwasser und Folgefrüchte. In: Ende der Nische, Beiträge zur 8. Wissenschaftstagung Ökologischer Landbau

Drinkwater LE, Snapp SS (2007) Nutrients in agroecosystems: rethinking the management paradigm. In: Sparks DL (ed) Advances in Agronomy. Academic Press, pp 163–186

Dube E, Chiduza C, Muchaonyerwa P (2014) High biomass yielding winter cover crops can improve phosphorus availability in soil. South Afr J Sci 110:01–04. https://doi.org/10.1590/sajs.2014/20130135

Durán Zuazo VH, Rodríguez Pleguezuelo CR (2008) Soil-erosion and runoff prevention by plant covers. A review. Agron Sustain Dev 28:65–86. https://doi.org/10.1051/agro:2007062

Eichler-Löbermann B, Gaj R, Schnug E (2009) Improvement of soil phosphorus availability by green fertilization with catch crops. Commun Soil Sci Plant Anal 40:70–81. https://doi.org/10.1080/00103620802623612

Eichler-Löbermann B, Köhne S, Kowalski B, Schnug E (2008) Effect of catch cropping on phosphorus bioavailability in comparison to organic and inorganic fertilization. J Plant Nutr 31:659–676. https://doi.org/10.1080/01904160801926517

Elbon A, Whalen JK (2015) Phosphorus supply to vegetable crops from arbuscular mycorrhizal fungi: a review. Biol Agric Hortic 31:73–90. https://doi.org/10.1080/01448765.2014.966147

Elfstrand S, Båth B, Mårtensson A (2007) Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. Appl Soil Ecol 36:70–82. https://doi.org/10.1016/j.apsoil.2006.11.001

Fageria NK, Gheyi HR, Carvalho MCS, Moreira A (2016) Root growth, nutrient uptake and use efficiency by roots of tropical legume cover crops as influenced by phosphorus fertilization. J Plant Nutr 39:781–792. https://doi.org/10.1080/01904167.2015.1088020

FAO (2021) FAO Director Qu Dongyu - Welcome address and opening remarks at the 8th World Congress on Conservation Agriculture. Bern

Faucon M-P, Houben D, Lambers H (2017) Plant functional traits: Soil and ecosystem services. Trends Plant Sci 22:385–394. https://doi.org/10.1016/j.tplants.2017.01.005

Faucon M-P, Houben D, Reynoird J-P, et al (2015) Advances and perspectives to improve the phosphorus availability in cropping systems for agroecological phosphorus management. In: Sparks DL (ed) Advances in Agronomy. Elsevier, New York, pp 51–79

Fester T, Sawers R (2011) Progress and challenges in agricultural applications of arbuscular mycorrhizal fungi. Crit Rev Plant Sci 30:459–470. https://doi.org/10.1080/07352689.2011.605741

Finckh M, Junge S, Schmidt J, Weedon O (2019) Disease and pest management in organic farming: a case for applied agroecology. In: Köpke U (ed) Improving organic crop cultivation. Burleigh Dodds Science Publishing Limited, Cambridge, UK, pp 271–301

Flügge U-I, Häusler RE, Ludewig F, Gierth M (2011) The role of transporters in supplying energy to plant plastids. J Exp Bot 62:2381–2392. https://doi.org/10.1093/jxb/erq361

Flury M (2018) Agroecology has to become economical. Rural 21 - Int. J. Rural Dev. 21:22–23

Frąc M, Hannula SE, Bełka M, Jędryczka M (2018) Fungal biodiversity and their role in soil health. Front Microbiol 0: https://doi.org/10.3389/fmicb.2018.00707

Frąc M, Oszust K, Lipiec J (2012) Community level physiological profiles (CLPP), characterization and microbial activity of soil amended with dairy sewage sludge. Sensors 12:3253–3268. https://doi.org/10.3390/s120303253

Franchini JC, Pavan MA, Miyazawa M (2004) Redistribution of phosphorus in soil through cover crop roots. Braz Arch Biol Technol 47:381–386. https://doi.org/10.1590/S1516-89132004000300007

Fraser TD, Lynch DH, Gaiero J, et al (2017) Quantification of bacterial non-specific acid (*phoC*) and alkaline (*phoD*) phosphatase genes in bulk and rhizosphere soil from organically managed soybean fields. Appl Soil Ecol 111:48–56. https://doi.org/10.1016/j.apsoil.2016.11.013

Frey SD, Elliott ET, Paustian K (1999) Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biol Biochem 31:573–585. https://doi.org/10.1016/S0038-0717(98)00161-8

Frey SD, Six J, Elliott ET (2003) Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil–litter interface. Soil Biol Biochem 35:1001–1004. https://doi.org/10.1016/S0038-0717(03)00155-X

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36. https://doi.org/10.1111/j.1469-8137.2007.02191.x

Frossard E, Condron LM, Oberson A, et al (2000) Processes governing phosphorus availability in temperate soils. J Environ Qual 29:15–23. https://doi.org/10.2134/jeq2000.00472425002900010003x

Frostegård A, Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol Fertil Soils 22:59–65. https://doi.org/10.1007/BF00384433

Frostegård Å, Bååth E, Tunlio A (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol Biochem 25:723–730. https://doi.org/10.1016/0038-0717(93)90113-P

Furutani H, Marumaya H, Wasaki J, et al (2017) White lupin root-secreted phosphatase and organic acids have a synergistic effect to mobilize unavailable phosphorus in soil. In: In Proceedings of XVIII International Plant Nutrition Colloquium. University of Copenhagen, Copenhagen (Denmark), pp 707–708

Gagolewski M (2018) R package stringi: Character string processing facilities. Version 1.4.3URL http://www.gagolewski.com/software/stringi/

Gaind S, Nain L (2015) Soil–phosphorus mobilization potential of phytate mineralizing fungi. J Plant Nutr 38:2159–2175. https://doi.org/10.1080/01904167.2015.1014561

Galvez L, Douds DD, Wagoner P, et al (1995) An overwintering cover crop increases inoculum of VAM fungi in agricultural soil. Am J Altern Agric 10:152–156. https://doi.org/10.1017/S0889189300006391

García-González I, Quemada M, Gabriel JL, et al (2018) Legacy of eight-year cover cropping on mycorrhizae, soil, and plants. J Plant Nutr Soil Sci 181:818–826. https://doi.org/10.1002/jpln.201700591

Gardner WK, Barber DA, Parbery DG (1983) The acquisition of phosphorus by *Lupinus albus* L. Plant Soil 70:107–124. https://doi.org/10.1007/BF02374754

Gardner WK, Boundy KA (1983) The acquisition of phosphorus by *Lupinus albus* L. IV. The effect of interplanting wheat and white lupin on the growth and mineral composition of the two species. Plant Soil 70:391–402

Garibay SV, Stamp P, Ammon HU, Feil B (1997) Yield and quality components of silage maize in killed and live cover crop sods. Eur J Agron 6:179–190. https://doi.org/10.1016/S1161-0301(96)02043-6

Garnier E, Lavorel S, Ansquer P, et al (2007) Assessing the effects of land-use change on plant traits, communities and ecosystem functioning in grasslands: a standardized methodology and lessons from an application to 11 European sites. Ann Bot 99:967–985. https://doi.org/10.1093/aob/mcl215

Garnier S (2018) viridis: default color maps from “matplotlib.” Version 0.5.1URL https://CRAN.R-project.org/package=viridis

Gavito ME, Olsson PA (2003) Allocation of plant carbon to foraging and storage in arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 45:181–187. https://doi.org/10.1016/S0168-6496(03)00150-8

Geleta SB, Briand CH, Womack HE, et al (2006) Is phosphorus fertilization necessary for watermelon production on high phosphorus soils? Can J Plant Sci 86:205–211. https://doi.org/10.4141/P04-093

George TS, Giles CD, Menezes-Blackburn D, et al (2018) Organic phosphorus in the terrestrial environment: a perspective on the state of the art and future priorities. Plant Soil 427:191–208. https://doi.org/10.1007/s11104-017-3391-x

Gerke J (2010) Humic (organic matter)-Al(Fe)-phosphate complexes: an underestimated phosphate form in soils and source of plant-available phosphate. Soil Sci 175:417–425. https://doi.org/10.1097/SS.0b013e3181f1b4dd

Gerke J (2015) Phytate (inositol hexakisphosphate) in soil and phosphate acquisition from inositol phosphates by higher plants. a review. Plants 4:253–266. https://doi.org/10.3390/plants4020253

Gerke J, Meyer U (1995) Phosphate aquisition by red clover and black mustard on a humic Podzol. J Plant Nutr 18:2409–2429. https://doi.org/10.1080/01904169509365074

Giaveno C, Celi L, Richardson AE, et al (2010) Interaction of phytases with minerals and availability of substrate affect the hydrolysis of inositol phosphates. Soil Biol Biochem 42:491–498. https://doi.org/10.1016/j.soilbio.2009.12.002

Gichangi EM, Njarui DMG, Gatheru MJ, et al (2016) Effects of brachiaria grasses on soil microbial biomass carbon, nitrogen and phosphorus in soils of the semi arid tropics of Kenia. Trop Subtrop Agroecosystems 19:193–203

Giles CD, Cade-Menun BJ, Hill JE (2011) The inositol phosphates in soils and manures: Abundance, cycling, and measurement. Can J Soil Sci 91:397–416. https://doi.org/10.4141/cjss09090

Giles CD, George TS, Brown LK, et al (2017) Linking the depletion of rhizosphere phosphorus to the heterologous expression of a fungal phytase in Nicotiana tabacum as revealed by enzyme-labile P and solution ^{31}P NMR spectroscopy. Rhizosphere 3:82–91. https://doi.org/10.1016/j.rhisph.2016.11.004

Gill RA, Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. New Phytol 147:13–31. https://doi.org/10.1046/j.1469-8137.2000.00681.x

Giovannetti M, Turrini A, Strani P, et al (2006) Mycorrhizal fungi in ecotoxicological studies: Soil impact of fungicides, insecticides and herbicides. Prev Today 2:47–62

Gomez E, Ferreras L, Toresani S (2006) Soil bacterial functional diversity as influenced by organic amendment application. Bioresour Technol 97:1484–1489. https://doi.org/10.1016/j.biortech.2005.06.021

Grant CA, Flaten DN, Tomasiewicz DJ, Sheppard SC (2001) The importance of early season phosphorus nutrition. Can J Plant Sci 81:211–224. https://doi.org/10.4141/P00-093

Groffman PM, Hendrix PF, Crossley DA (1987) Effects of a winter legume on phosphorus, potassium, calcium and magnesium cycling in a humid subtropical agroecosystem. Agric Ecosyst Environ 18:281–289. https://doi.org/10.1016/0167-8809(87)90095-8

Gryta A, Frąc M, Oszust K (2014) The application of the Biolog Ecoplate approach in ecotoxicological evaluation of dairy sewage sludge. Appl Biochem Biotechnol 174:1434–1443. https://doi.org/10.1007/s12010-014-1131-8

Guggenberger G, Haumaier L, Zech W, Thomas RJ (1996) Assessing the organic phosphorus status of an Oxisol under tropical pastures following native savanna using ^{31}P NMR spectroscopy. Biol Fertil Soils 23:332–339. https://doi.org/10.1007/BF00335963

Guhr A, Borken W, Spohn M, Matzner E (2015) Redistribution of soil water by a saprotrophic fungus enhances carbon mineralization. Proc Natl Acad Sci 112:14647–14651. https://doi.org/10.1073/pnas.1514435112

Guo F, Yost RS, Hue NV, et al (2000) Changes in phosphorus fractions in soils under intensive plant growth. Soil Sci Soc Am J 64:1681–1689

Guppy CN, Menzies NW, Moody PW, Blamey FPC (2005) Competitive sorption reactions between phosphorus and organic matter in soil: a review. Soil Res 43:189–202. https://doi.org/10.1071/SR04049

Halekoh U, Højsgaard S (2014) A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models – the R package pbkrtest. J Stat Softw 59:1–30

Hallama M, Pekrun C, Lambers H, Kandeler E (2019) Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. Plant Soil 434:7–45. https://doi.org/10.1007/s11104-018-3810-7

Hallama M, Pekrun C, Pilz S, et al (2021) Interactions between cover crops and soil microorganisms increase phosphorus availability in conservation agriculture. Plant Soil 463:307–328. https://doi.org/10.1007/s11104-021-04897-x

Harold FM (1966) Inorganic polyphosphates in biology: structure, metabolism, and function. Bacteriol Rev 30:772–794

Harrison AF (1987) Soil organic phosphorus: a review of world literature. Commonwealth Agricultural Bureaux International, Wallingford, Oxon

Hayat R, Ali S, Amara U, et al (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598. https://doi.org/10.1007/s13213-010-0117-1

He ZL, Wu J, O'Donnell AG, Syers JK (1997) Seasonal responses in microbial biomass carbon, phosphorus and sulphur in soils under pasture. Biol Fertil Soils 24:421–428. https://doi.org/10.1007/s003740050267

Hedley MJ, Stewart JWB, Chauhan BS (1982) Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci Soc Am J 46:970–976

Heiden E (1865) Die Phosphorsäure in ihren Beziehungen zur Landwirthschaft, G. Grote'sche Verlagsbuchhandlung. Hamm

Higo M, Isobe K, Drijber RA, et al (2014) Impact of a 5-year winter cover crop rotational system on the molecular diversity of arbuscular mycorrhizal fungi colonizing roots of subsequent soybean. Biol Fertil Soils 50:913–926. https://doi.org/10.1007/s00374-014-0912-0

Hill JE, Richardson AE (2007) Isolation and assessment of microorganisms that utilize phytate. In: Turner BL, Richardson AE, Mullaney EJ (eds) Inositol phosphates: linking agriculture and the environment. CABI, Wallingford, pp 61–77

Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil 237:173–195. https://doi.org/10.1023/A:1013351617532

Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321:117–152. https://doi.org/10.1007/s11104-008-9885-9

Hinsinger P, Brauman A, Devau N, et al (2011) Acquisition of phosphorus and other poorly mobile nutrients by roots. Where do plant nutrition models fail? Plant Soil 348:29–61. https://doi.org/10.1007/s11104-011-0903-y

Hinsinger P, Herrmann L, Lesueur D, et al (2015) Impact of roots, microorganisms and microfauna on the fate of soil phosphorus in the rhizosphere. In: Plaxton W, Lambers H (eds) Phosphorus Metabolism of Plants. John Wiley & Sons, pp 377–407

Hinsinger P, Plassard C, Tang C, Jaillard B (2003) Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil 248:43–59. https://doi.org/10.1023/A:1022371130939

Ho A, Di Lonardo DP, Bodelier PLE (2017) Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiol Ecol 93:1–14. https://doi.org/10.1093/femsec/fix006

Hobbs PR, Sayre K, Gupta R (2008) The role of conservation agriculture in sustainable agriculture. Philos Trans R Soc B Biol Sci 363:543–555. https://doi.org/10.1098/rstb.2007.2169

Hoffland E, Van Den Boogaard R, Nelemans J, Findenegg G (1992) Biosynthesis and root exudation of citric and malic acids in phosphate-starved rape plants. New Phytol 122:675–680. https://doi.org/10.1111/j.1469-8137.1992.tb00096.x

Høgh-Jensen H, Schjørring JK (1997) Interactions between white clover and ryegrass under contrasting nitrogen availability: N_2 fixation, N fertilizer recovery, N transfer and water use efficiency. Plant Soil 197:187–199

Honvault N, Houben D, Firmin S, et al (2021) Interactions between below-ground traits and rhizosheath fungal and bacterial communities for phosphorus acquisition. Funct Ecol 35:1603–1619. https://doi.org/10.1111/1365-2435.13823

Honvault N, Houben D, Nobile C, et al (2020) Tradeoffs among phosphorus-acquisition root traits of crop species for agroecological intensification. Plant Soil 461:137–150. https://doi.org/10.1007/s11104-020-04584-3

Horst WJ, Kamh M, Jibrin JM, Chude VO (2001) Agronomic measures for increasing P availability to crops. Plant Soil 237:211–223

Horst WJ, Waschkies Ch (1987) Phosphatversorgung von Sommerweizen (*Triticum aestivum* L.) in Mischkultur mit Weißer Lupine (*Lupinus albus* L.). Z Für Pflanzenernähr Bodenkd 150:1–8. https://doi.org/10.1002/jpln.19871500102

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–363

Hunter PJ, Teakle GR, Bending GD (2014) Root traits and microbial community interactions in relation to phosphorus availability and acquisition, with particular reference to Brassica. Front Plant Sci 5:1–18. https://doi.org/10.3389/fpls.2014.00027

IAASTD (2009) International assessment of agricultural knowledge, science and technology for development (IAASTD) : global report. Island Press, Washington, DC

Ikerra ST, Semu E, Mrema JP (2006) Combining *Tithonia diversifolia* and minjingu phosphate rock for improvement of P availability and maize grain yields on a chromic acrisol in Morogoro, Tanzania. Nutr Cycl Agroecosystems 76:249–260. https://doi.org/10.1007/s10705-006-9007-0

Insam H (1997) A new set of substrates proposed for community characterization in environmental samples. In: Insam H, Rangger A (eds) Microbial Communities. Springer, Berlin, Heidelberg, pp 259–260

Insam H, Goberna M (2004) Use of biolog for the community level physiological profiling (CLPP) of environmental samples. Mol Microb Ecol Man 4.01:853–860

IUSS Working Group WRB (2015) World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. FAO, Rome

Iyamuremye F, Dick RP, Baham J (1996) Organic amendments and phosphorus dynamics: II. distribution of soil phosphorus fractions. Soil Sci 161:436–443

Jacoby R, Peukert M, Succurro A, et al (2017) The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. Front Plant Sci 8:1617. https://doi.org/10.3389/fpls.2017.01617

Janegitz MC, Inoue BS, Rosolem CA (2013) Soil phosphorus pools as affected by brachiaria and white lupin. Cienc Rural 43:1381–1386. https://doi.org/10.1590/S0103-84782013000800007

Jansa J, Finlay R, Wallander H, et al (2011) Role of mycorrhizal symbioses in phosphorus cycling. In: Bünemann E, Oberson A, Frossard E (eds) Phosphorus in Action. Springer, Berlin, Heidelberg, pp 137–168

Jansa J, Mozafar A, Kuhn G, et al (2003) Soil tillage affects the community structure of mycorrhizal fungi in maize roots. Ecol Appl 13:1164–1176. https://doi.org/10.1890/1051-0761

Jarosch KA, Doolette AL, Smernik RJ, et al (2015) Characterisation of soil organic phosphorus in NaOH-EDTA extracts: A comparison of ^{31}P NMR spectroscopy and enzyme addition assays. Soil Biol Biochem 91:298–309. https://doi.org/10.1016/j.soilbio.2015.09.010

Jarosch KA, Kandeler E, Frossard E, Bünemann EK (2019) Is the enzymatic hydrolysis of soil organic phosphorus compounds limited by enzyme or substrate availability? Soil Biol Biochem 139:107628. https://doi.org/10.1016/j.soilbio.2019.107628

Jemo M, Abaidoo RC, Nolte C, et al (2006) Phosphorus benefits from grain-legume crops to subsequent maize grown on acid soils of southern Cameroon. Plant Soil 284:385–397. https://doi.org/10.1007/s11104-006-0052-x

Jensen LS, Pedersen A, Magid J, Nielsen NE (2005) Catch crops have little effect on P and K availability of depleted soils. DARCOF E-News 2:1–7

Johal GS, Huber DM (2009) Glyphosate effects on diseases of plants. Eur J Agron 31:144–152. https://doi.org/10.1016/j.eja.2009.04.004

Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytol 135:575–585

Johnston AM, Bruulsema TW (2014) 4R nutrient stewardship for improved nutrient use efficiency. Procedia Eng 83:365–370. https://doi.org/10.1016/j.proeng.2014.09.029

Joner EJ, Aarle IM van, Vosatka M (2000) Phosphatase activity of extra-radical arbuscular mycorrhizal hyphae: A review. Plant Soil 226:199–210

Jones DL (1998) Organic acids in the rhizosphere – a critical review. Plant Soil 205:25–44. https://doi.org/10.1023/A:1004356007312

Jones DL, Edwards AC (1998) Influence of sorption on the biological utilization of two simple carbon substrates. Soil Biol Biochem 30:1895–1902. https://doi.org/10.1016/S0038-0717(98)00060-1

Jones DL, Oburger E (2011) Solubilization of phosphorus by soil microorganisms. In: Bünemann E, Oberson A, Frossard E (eds) Phosphorus in Action. Springer, Berlin, Heidelberg, pp 169–198

Jorquera MA, Hernández MT, Rengel Z, et al (2008) Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. Biol Fertil Soils 44:1025. https://doi.org/10.1007/s00374-008-0288-0

Junge SM, Storch J, Finckh MR, Schmidt JH (2020) Developing organic minimum tillage farming systems for Central and Northern European conditions. In: Dang YP, Dalal RC, Menzies NW (eds) No-till Farming Systems for Sustainable Agriculture: Challenges and Opportunities. Springer International Publishing, Cham, pp 173–192

Kabir Z (2005) Tillage or no-tillage: Impact on mycorrhizae. Can J Plant Sci 85:23–29. https://doi.org/10.4141/P03-160

Kabir Z, Koide RT (2002) Effect of autumn and winter mycorrhizal cover crops on soil properties, nutrient uptake and yield of sweet corn in Pennsylvania, USA. Plant Soil 238:205–215

Kabir Z, Koide RT (2000) The effect of dandelion or a cover crop on mycorrhiza inoculum potential, soil aggregation and yield of maize. Agric Ecosyst Environ 78:167–174. https://doi.org/10.1016/S0167-8809(99)00121-8

Kabiri V, Raiesi F, Ghazavi MA (2016) Tillage effects on soil microbial biomass, SOM mineralization and enzyme activity in a semi-arid Calcixerepts. Agric Ecosyst Environ 232:73–84. https://doi.org/10.1016/j.agee.2016.07.022

Kamh M, Abdou M, Chude V, et al (2002) Mobilization of phosphorus contributes to positive rotational effects of leguminous cover crops on maize grown on soils from northern Nigeria. J Plant Nutr Soil Sci 165:566–572. https://doi.org/10.1002/1522-2624(200210)165:5<566::AID-JPLN566>3.0.CO;2-O

Kamh M, Horst WJ, Chude VO (1998) Mobilization of soil and fertilizer phosphate by cover crops. In: Merbach W (ed) Pflanzenernährung, Wurzelleistung und Exsudation. Vieweg+Teubner Verlag, Leipzig, pp 167–177

Kandeler E (1990) Characterization of free and adsorbed phosphatases in soils. Biol Fertil Soils 9:199–202. https://doi.org/10.1007/BF00335808

Kandeler E, Mosier AR, Morgan JA, et al (2006) Response of soil microbial biomass and enzyme activities to the transient elevation of carbon dioxide in a semi-arid grassland. Soil Biol Biochem 38:2448–2460. https://doi.org/10.1016/j.soilbio.2006.02.021

Kanter DR, Brownlie WJ (2019) Joint nitrogen and phosphorus management for sustainable development and climate goals. Environ Sci Policy 92:1–8. https://doi.org/10.1016/j.envsci.2018.10.020

Karasawa T, Takahashi S (2015) Introduction of various cover crop species to improve soil biological P parameters and P uptake of the following crops. Nutr Cycl Agroecosystems 103:15–28. https://doi.org/10.1007/s10705-015-9715-4

Karasawa T, Takebe M (2012) Temporal or spatial arrangements of cover crops to promote arbuscular mycorrhizal colonization and P uptake of upland crops grown after

nonmycorrhizal crops. Plant Soil 353:355–366. https://doi.org/10.1007/s11104-011-1036-z

Kaspar TC, Singer JW (2015) The use of cover crops to manage soil. In: Hatfield JL, Sauer TJ (eds) Soil Management: Building a Stable Base for Agriculture. Soil Science Society of America, Madison, WI, USA, pp 321–337

Kaye JP, Quemada M (2017) Using cover crops to mitigate and adapt to climate change. A review. Agron Sustain Dev 37:4. https://doi.org/10.1007/s13593-016-0410-x

Keller M, Oberson A, Annaheim KE, et al (2012) Phosphorus forms and enzymatic hydrolyzability of organic phosphorus in soils after 30 years of organic and conventional farming. J Plant Nutr Soil Sci 175:385–393. https://doi.org/10.1002/jpln.201100177

Kelly JB, Midgley AR (1943) Phosphate fixation -An exchange of phosphate and hydroxyl ions. Soil Sci 55:167–176

Kim N, Zabaloy MC, Guan K, Villamil MB (2020a) Do cover crops benefit soil microbiome? A meta-analysis of current research. Soil Biol Biochem 142:107701. https://doi.org/10.1016/j.soilbio.2019.107701

Kim N, Zabaloy MC, Riggins CW, et al (2020b) Microbial shifts following five years of cover cropping and tillage practices in fertile agroecosystems. Microorganisms 8:1773. https://doi.org/10.3390/microorganisms8111773

Kirchmann H, Wessling J (2017) Kinetics of inorganic and organic P release from red clover (*Trifolium pratense* L.) and ryegrass (*Lolium multiflorum* L.) upon frost or drying. Acta Agric Scand Sect B — Soil Plant Sci 67:693–696. https://doi.org/10.1080/09064710.2017.1339823

Kleinman PJ, Salon P, Sharpley AN (2001) Evaluating alternative cover crops for the control of runoff phosphorus losses. In: 2001 Annual meetings abstracts. ASA, CSSA, and SSSA, Madison, WI

Knapp S, van der Heijden MGA (2018) A global meta-analysis of yield stability in organic and conservation agriculture. Nat Commun 9:3632. https://doi.org/10.1038/s41467-018-05956-1

Koide RT, Mosse B (2004) A history of research on arbuscular mycorrhiza. Mycorrhiza 14:145–163. https://doi.org/10.1007/s00572-004-0307-4

Koné AW, Tondoh JE, Angui PKT, et al (2008) Is soil quality improvement by legume cover crops a function of the initial soil chemical characteristics? Nutr Cycl Agroecosystems 82:89–105. https://doi.org/10.1007/s10705-008-9172-4

Konietzny U, Greiner R (2002) Molecular and catalytic properties of phytate-degrading enzymes (phytases). Int J Food Sci Technol 37:791–812. https://doi.org/10.1046/j.1365-2621.2002.00617.x

Kouno K, Tuchiya Y, Ando T (1995) Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biol Biochem 27:1353–1357. https://doi.org/10.1016/0038-0717(95)00057-L

Kozak M, Piepho H-P (2018) What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. J Agron Crop Sci 204:86–98. https://doi.org/10.1111/jac.12220

Kramer S, Marhan S, Haslwimmer H, et al (2013) Temporal variation in surface and subsoil abundance and function of the soil microbial community in an arable soil. Soil Biol Biochem 61:76–85. https://doi.org/10.1016/j.soilbio.2013.02.006

Kramer S, Marhan S, Ruess L, et al (2012) Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems. 55:

Kumar D, Shivay YS, Dhar S, et al (2013) Rhizospheric flora and the influence of agronomic practices on them: A review. Proc Natl Acad Sci India Sect B - Biol Sci 83:1–14. https://doi.org/10.1007/s40011-012-0059-4

Kunze A, Costa MD, Epping J, et al (2011) Phosphatase activity in sandy soil influenced by mycorrhizal and non-mycorrhizal cover crops. Rev Bras Ciênc Solo 35:705–711. https://doi.org/10.1590/S0100-06832011000300005

Kuo S, Huang B, Bembenek R (2005) Effects of long-term phosphorus fertilization and winter cover cropping on soil phosphorus transformations in less weathered soil. Biol Fertil Soils 41:116–123. https://doi.org/10.1007/s00374-004-0807-6

Kuznetsova A, Brockhoff P, Christensen R (2017) lmerTest package: tests in linear mixed effects models. J Stat Softw Artic 82:1–26. https://doi.org/10.18637/jss.v082.i13

Kuznetsova A, Brockhoff PB, Christensen RHB (2016) lmerTest: tests in linear mixed effects models. Version 2.0-33URL https://CRAN.R-project.org/package=lmerTest

Lal R, Wilson GF, Okigbo BN (1978) No-till farming after various grasses and leguminous cover crops in tropical Alfisol. I. Crop performance. Field Crops Res 1:71–84

Lambers H, Albornoz F, Kotula L, et al (2018) How belowground interactions contribute to the coexistence of mycorrhizal and non-mycorrhizal species in severely phosphorus-impoverished hyperdiverse ecosystems. Plant Soil 424:11–33. https://doi.org/10.1007/s11104-017-3427-2

Lambers H, Clements JC, Nelson MN (2013) How a phosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (*Lupinus*, Fabaceae). Am J Bot 100:263–288. https://doi.org/10.3732/ajb.1200474

Lambers H, Clode PL, Hawkins H-J, et al (2015a) Metabolic adaptations of the non-mycotrophic Proteaceae to soils with low phosphorus availability. In: Plaxton WC, Lambers H (eds) Phosphorus Metabolism in Plants. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp 289–335

Lambers H, Hayes PE, Laliberté E, et al (2015b) Leaf manganese accumulation and phosphorus-acquisition efficiency. Trends Plant Sci 20:83–90. https://doi.org/10.1016/j.tplants.2014.10.007

Lambers H, Shane MW, Cramer MD, et al (2006) Root Structure and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and Physiological Traits. Ann Bot 98:693–713. https://doi.org/10.1093/aob/mcl114

Lambers H, Teste FP (2013) Interactions between arbuscular mycorrhizal and non-mycorrhizal plants: do non-mycorrhizal species at both extremes of nutrient availability play the same game? Plant Cell Environ 36:1911–1915. https://doi.org/10.1111/pce.12117

Lang F, Krüger J, Amelung W, et al (2017) Soil phosphorus supply controls P nutrition strategies of beech forest ecosystems in Central Europe. Biogeochemistry 136:5–29. https://doi.org/10.1007/s10533-017-0375-0

Lass D, Bevis A, Stevenson GW, et al (2003) Community supported agriculture entering the 21st century: Results from the 2001 national survey. University of Massachusetts, Department of Resource Economics, Amherst

Lehman RM, Taheri WI, Osborne SL, et al (2012) Fall cover cropping can increase arbuscular mycorrhizae in soils supporting intensive agricultural production. Appl Soil Ecol 61:300–304. https://doi.org/10.1016/j.apsoil.2011.11.008

LeMare P, Pereira J, Goedert W (1987) Effects of green manure on isotopically exchangeable phosphate in a dark-red latosol in Brazil. J Soil Sci 38:199–209

Lemming C, Oberson A, Magid J, et al (2019) Residual phosphorus availability after long-term soil application of organic waste. Agric Ecosyst Environ 270–271:65–75. https://doi.org/10.1016/j.agee.2018.10.009

Lenth R (2018) emmeans: estimated marginal means, aka least-squares means. Version 1.1URL https://CRAN.R-project.org/package=emmeans

Li B, Li Y-Y, Wu H-M, et al (2016) Root exudates drive interspecific facilitation by enhancing nodulation and N2 fixation. Proc Natl Acad Sci 113:6496–6501. https://doi.org/10.1073/pnas.1523580113

Li L, Li S-M, Sun J-H, et al (2007) Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. Proc Natl Acad Sci 104:11192–11196. https://doi.org/10.1073/pnas.0704591104

Li L, Tilman D, Lambers H, Zhang F-S (2014) Plant diversity and overyielding: insights from belowground facilitation of intercropping in agriculture. New Phytol 203:63–69. https://doi.org/10.1111/nph.12778

Li X, Garbeva P, Liu X, et al (2020) Volatile-mediated antagonism of soil bacterial communities against fungi. Environ Microbiol 22:1025–1035. https://doi.org/10.1111/1462-2920.14808

Little SA, Hocking PJ, Greene RSB (2004) A preliminary study of the role of cover crops in improving soil fertility and yield for potato production. Commun Soil Sci Plant Anal 35:471–494. https://doi.org/10.1081/CSS-120029726

Liu J, Bergkvist G, Ulén B (2015) Biomass production and phosphorus retention by catch crops on clayey soils in southern and central Sweden. Field Crops Res 171:130–137. https://doi.org/10.1016/j.fcr.2014.11.013

López-Gutiérrez JC, Henry S, Hallet S, et al (2004) Quantification of a novel group of nitrate-reducing bacteria in the environment by real-time PCR. J Microbiol Methods 57:399–407. https://doi.org/10.1016/j.mimet.2004.02.009

Lyu Y, Tang H, Li H, et al (2016) Major crop species show differential balance between root morphological and physiological responses to variable phosphorus supply. Front Plant Sci 7:1939. https://doi.org/10.3389/fpls.2016.01939

Ma X-F, Wright E, Ge Y, et al (2009) Improving phosphorus acquisition of white clover (*Trifolium repens* L.) by transgenic expression of plant-derived phytase and acid phosphatase genes. Plant Sci 176:479–488. https://doi.org/10.1016/j.plantsci.2009.01.001

Maltais-Landry G (2015) Legumes have a greater effect on rhizosphere properties (pH, organic acids and enzyme activity) but a smaller impact on soil P compared to other cover crops. Plant Soil 394:139–154. https://doi.org/10.1007/s11104-015-2518-1

Maltais-Landry G, Frossard E (2015) Similar phosphorus transfer from cover crop residues and water-soluble mineral fertilizer to soils and a subsequent crop. Plant Soil 393:193–205. https://doi.org/10.1007/s11104-015-2477-6

Maltais-Landry G, Scow K, Brennan E (2014) Soil phosphorus mobilization in the rhizosphere of cover crops has little effect on phosphorus cycling in California agricultural soils. Soil Biol Biochem 78:255–262. https://doi.org/10.1016/j.soilbio.2014.08.013

Maltais-Landry G, Scow K, Brennan E, Vitousek P (2015) Long-term effects of compost and cover crops on soil phosphorus in two California agroecosystems. Soil Sci Soc Am J 79:688–697. https://doi.org/10.2136/sssaj2014.09.0369

Mamy L, Barriuso E, Gabrielle B (2016) Glyphosate fate in soils when arriving in plant residues. Chemosphere 154:425–433. https://doi.org/10.1016/j.chemosphere.2016.03.104

Manna MC, Rao AS, Ganguly TK (2007) Effect of fertilizer p and farmyard manure on bioavailable p as influenced by rhizosphere microbial activities in soybean-wheat rotation. J Sustain Agric 29:149–166. https://doi.org/10.1300/J064v29n03_12

Marilley L, Aragno M (1999) Phylogenetic diversity of bacterial communities differing in degree of proximity of *Lolium perenne* and *Trifolium repens* roots. Appl Soil Ecol 13:127–136. https://doi.org/10.1016/S0929-1393(99)00028-1

Marques E, Kur A, Bueno E, Wettberg E von (2020) Defining and improving the rotational and intercropping value of a crop using a plant–soil feedbacks approach. Crop Sci 60:2195–2203. https://doi.org/10.1002/csc2.20200

Marschener H (1998) Role of root growth, arbuscular mycorrhiza, and root exudates for the efficiency in nutrient acquisition. Field Crops Res 56:203–207. https://doi.org/10.1016/S0378-4290(97)00131-7

Marschner P, Yang C-H, Lieberei R, Crowley DE (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem 33:1437–1445. https://doi.org/10.1016/S0038-0717(01)00052-9

Marx M-C, Wood M, Jarvis SC (2001) A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biol Biochem 33:1633–1640. https://doi.org/10.1016/S0038-0717(01)00079-7

Mason SD, McLaughlin MJ, Johnston C, McNeill A (2013) Soil test measures of available P (Colwell, resin and DGT) compared with plant P uptake using isotope dilution. Plant Soil 373:711–722. https://doi.org/10.1007/s11104-013-1833-7

Mat Hassan H, Hasbullah H, Marschner P (2013) Growth and rhizosphere P pools of legume-wheat rotations at low P supply. Biol Fertil Soils 49:41–49. https://doi.org/10.1007/s00374-012-0695-0

Maul J, Drinkwater L (2010) Short-term plant species impact on microbial community structure in soils with long-term agricultural history. Plant Soil 330:369–382. https://doi.org/10.1007/s11104-009-0211-y

Maul JE, Buyer JS, Lehman RM, et al (2014) Microbial community structure and abundance in the rhizosphere and bulk soil of a tomato cropping system that includes cover crops. Appl Soil Ecol 77:42–50. https://doi.org/10.1016/j.apsoil.2014.01.002

Mbuthia LW, Acosta-Martínez V, DeBruyn J, et al (2015) Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. Soil Biol Biochem 89:24–34. https://doi.org/10.1016/j.soilbio.2015.06.016

McClain ME, Boyer EW, Dent CL, et al (2003) Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301–312. https://doi.org/10.1007/s10021-003-0161-9

McCourty MA, Gyawali AJ, Stewart RD (2018) Of macropores and tillage: influence of biomass incorporation on cover crop decomposition and soil respiration. Soil Use Manag 34:101–110. https://doi.org/10.1111/sum.12403

McLaren TI, Smernik RJ, McLaughlin MJ, et al (2020) Chapter Two - The chemical nature of soil organic phosphorus: A critical review and global compilation of quantitative data. In: Sparks DL (ed) Advances in Agronomy. Academic Press, pp 51–124

McLaren TI, Smernik RJ, McLaughlin MJ, et al (2015) Complex forms of soil organic phosphorus–A major component of soil phosphorus. Environ Sci Technol 49:13238–13245. https://doi.org/10.1021/acs.est.5b02948

McLaughlin MJ, Alston AM, Martin JK (1986) Measurement of phosphorus in the soil microbial biomass: A modified procedure for field soils. Soil Biol Biochem 18:437–443. https://doi.org/10.1016/0038-0717(86)90050-7

McLaughlin MJ, McBeath TM, Smernik R, et al (2011) The chemical nature of P accumulation in agricultural soils—implications for fertiliser management and design: an Australian perspective. Plant Soil 349:69–87. https://doi.org/10.1007/s11104-011-0907-7

Medhi DN, Datta SKD (1996) Phosphorus availability to irrigated lowland rice as affected by sources, application level and green manure. Nutr Cycl Agroecosystems 46:195–203. https://doi.org/10.1007/BF00420554

Mehlich A (1984) Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun Soil Sci Plant Anal 15:1409–1416. https://doi.org/10.1080/00103628409367568

Menezes-Blackburn D, Giles C, Darch T, et al (2018) Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: a review. Plant Soil 427:5–16. https://doi.org/10.1007/s11104-017-3362-2

Menezes-Blackburn D, Jorquera MA, Greiner R, et al (2013) Phytases and Phytase-Labile Organic Phosphorus in Manures and Soils. Crit Rev Environ Sci Technol 43:916–954. https://doi.org/10.1080/10643389.2011.627019

Messiga AJ, Sharifi M, Munroe S (2016) Combinations of cover crop mixtures and bio-waste composts enhance biomass production and nutrients accumulation: a greenhouse

study. Renew Agric Food Syst 31:507–515. https://doi.org/10.1017/S1742170515000423

Miguez FE, Bollero GA (2005) Review of corn yield response under winter cover cropping systems using meta-analytic methods. Crop Sci 45:2318–2329. https://doi.org/10.2135/cropsci2005.0014

Miller M, Palojärvi A, Rangger A, et al (1998) The use of fluorogenic substrates to measure fungal presence and activity in soil. Appl Environ Microbiol 64:613–617

Mirsky SB, Ryan MR, Curran WS, et al (2012) Conservation tillage issues: Cover crop-based organic rotational no-till grain production in the mid-Atlantic region, USA. Renew Agric Food Syst 27:31–40. https://doi.org/10.1017/S1742170511000457

Moonen AC, Bàrberi P (2006) An ecological approach to study the physical and chemical effects of rye cover crop residues on *Amaranthus retroflexus*, *Echinochloa crus-galli* and maize. Ann Appl Biol 148:73–89. https://doi.org/10.1111/j.1744-7348.2006.00038.x

Mukumbareza C, Muchaonyerwa P, Chiduza C (2015) Effects of oats and grazing vetch cover crops and fertilisation on microbial biomass and activity after five years of rotation with maize. South Afr J Plant Soil 32:189–197. https://doi.org/10.1080/02571862.2015.1025446

Müller K (2020) here: a simpler way to find your files. Version 1.0.1URL https://CRAN.R-project.org/package=here

Müller K, Kubsch N, Marhan S, et al (2020) Saprotrophic and ectomycorrhizal fungi contribute differentially to organic p mobilization in beech-dominated forest ecosystems. Front For Glob Change 3:1–16. https://doi.org/10.3389/ffgc.2020.00047

Mulvaney MJ, Wood CW, Balkcom KS, et al (2017) No-till with high biomass cover crops and invasive legume mulches increased total soil carbon after three years of collard production. Agroecol Sustain Food Syst 41:30–45. https://doi.org/10.1080/21683565.2016.1236766

Muzangwa L, Chiduza C, Muchaonyerwa P (2012) Biomass production, weed suppression, nitrogen and phosphorus uptake in white oat (*Avena sativa* L.) and grazing vetch (*Vicia dasycarpa* L.) cover crop bicultures under an irrigated no-till system. South Afr J Plant Soil 29:135–141. https://doi.org/10.1080/02571862.2012.741719

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Murungu FS, Chiduza C, Muchaonyerwa P, Mnkeni PNS (2011a) Decomposition, nitrogen and phosphorus mineralization from winter-grown cover crop residues and

suitability for a smallholder farming system in South Africa. Nutr Cycl Agroecosystems 89:115–123. https://doi.org/10.1007/s10705-010-9381-5

Murungu FS, Chiduza C, Muchaonyerwa P, Mnkeni PNS (2011b) Mulch effects on soil moisture and nitrogen, weed growth and irrigated maize productivity in a warm-temperate climate of South Africa. Soil Tillage Res 112:58–65. https://doi.org/10.1016/j.still.2010.11.005

Nakazawa M (2018) fmsb: functions for medical statistics book with some demographic data. Version 0.6.3URL https://CRAN.R-project.org/package=fmsb

Nannipieri P, Ascher J, Ceccherini MT, et al (2008) Effects of root exudates in microbial diversity and activity in rhizosphere soils. In: Nautiyal PDCS, Dion PDP (eds) Molecular mechanisms of plant and microbe coexistence. Springer Berlin Heidelberg, pp 339–365

Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Role of phosphatase enzymes in soil. In: Bünemann E, Oberson A, Frossard E (eds) Phosphorus in Action. Springer Berlin Heidelberg, pp 215–243

Nascente AS, Crusciol CAC, Cobucci T, Velini ED (2013) Cover crop termination timing on rice crop production in a no-till system. Crop Sci 53:2659–2669. https://doi.org/10.2135/cropsci2013.01.0047

Ndour PMS, Heulin T, Achouak W, et al (2020) The rhizosheath: from desert plants adaptation to crop breeding. Plant Soil 456:1–13. https://doi.org/10.1007/s11104-020-04700-3

Negassa W, Leinweber P (2009) How does the Hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: A review. J Plant Nutr Soil Sci 172:305–325. https://doi.org/10.1002/jpln.200800223

Neill SP, Lee DR (2001) Explaining the adoption and disadoption of sustainable agriculture: the case of cover crops in northern Honduras. Econ Dev Cult Change 49:793–820

Nesme T, Withers PJA (2016) Sustainable strategies towards a phosphorus circular economy. Nutr Cycl Agroecosystems 104:259–264. https://doi.org/10.1007/s10705-016-9774-1

Neuwirth E (2014) RColorBrewer: ColorBrewer palettes. Version 1.1-2URL https://CRAN.R-project.org/package=RColorBrewer

Newman EI, Eason WR (1989) Cycling of nutrients from dying roots to living plants, including the role of mycorrhizas. Plant Soil 115:211–215

Ngosong C, Gabriel E, Ruess L (2012) Use of the signature fatty acid 16:1ω5 as a tool to determine the distribution of arbuscular mycorrhizal fungi in soil. J Lipids 8. http://dx.doi.org/10.1155/2012/236807

Njeru EM, Avio L, Sbrana C, et al (2014) First evidence for a major cover crop effect on arbuscular mycorrhizal fungi and organic maize growth. Agron Sustain Dev 34:841–848. https://doi.org/10.1007/s13593-013-0197-y

Nobile C, Houben D, Michel E, et al (2019) Phosphorus-acquisition strategies of canola, wheat and barley in soil amended with sewage sludges. Sci Rep 9:14878. https://doi.org/10.1038/s41598-019-51204-x

Nuruzzaman M, Lambers H, Bolland MD, Veneklaas EJ (2005a) Phosphorus uptake by grain legumes and subsequently grown wheat at different levels of residual phosphorus fertiliser. Crop Pasture Sci 56:1041–1047

Nuruzzaman M, Lambers H, Bolland MD, Veneklaas EJ (2005b) Phosphorus benefits of different legume crops to subsequent wheat grown in different soils of Western Australia. Plant Soil 271:175–187. https://doi.org/10.1007/s11104-004-2386-6

Nuruzzaman M, Lambers H, Bolland MDA, Veneklaas EJ (2006) Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. Plant Soil 281:109–120. https://doi.org/10.1007/s11104-005-3936-2

Oberson A, Bünemann E, Friesen DK, et al (2006) Improving phosphorus fertility in tropical soils through biological interventions. In: Uphoff N (ed) Biological Approaches to Sustainable Soil Systems, CRC Press. Boca Raton, pp 531–546

Oburger E, Jones DL, Wenzel WW (2011) Phosphorus saturation and pH differentially regulate the efficiency of organic acid anion-mediated P solubilization mechanisms in soil. Plant Soil 341:363–382. https://doi.org/10.1007/s11104-010-0650-5

Odhiambo JJO, Bomke AA (2007) Cover crop effects on spring soil water content and the implications for cover crop management in south coastal British Columbia. Agric Water Manag 88:92–98. https://doi.org/10.1016/j.agwat.2006.09.001

Oehl F, Oberson A, Probst M, et al (2001) Kinetics of microbial phosphorus uptake in cultivated soils. Biol Fertil Soils 34:31–41. https://doi.org/10.1007/s003740100362

Oehl F, Sieverding E, Ineichen K, et al (2003) Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central europe. Appl Environ Microbiol 69:2816–2824. https://doi.org/10.1128/AEM.69.5.2816-2824.2003

Oelmann Y, Lange M, Leimer S, et al (2021) Above- and belowground biodiversity jointly tighten the P cycle in agricultural grasslands. Nat Commun 12:4431. https://doi.org/10.1038/s41467-021-24714-4

Ohno T, Crannell BS (1996) Green and animal manure-derived dissolved organic matter effects on phosphorus sorption. J Environ Qual 25:1137–1143. https://doi.org/10.2134/jeq1996.00472425002500050029x

Ohno T, Zibilske LM (1991) Determination of low concentrations of phosphorus in soil extracts using malachite green. Soil Sci Soc Am J 55:892–895

Oikeh SO, Somado EA, Sahrawat KL, et al (2008) Rice yields enhanced through integrated management of cover crops and phosphate rock in phosphorus-deficient Ultisols in West Africa. Commun Soil Sci Plant Anal 39:2894–2919. https://doi.org/10.1080/00103620802432816

Oliveira RA de, Comin JJ, Tiecher T, et al (2017) Release of phosphorus forms from cover crop residues in agroecological no-till onion production. Rev Bras Ciênc Solo 41:1–16. https://doi.org/10.1590/18069657rbcs20160272

Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ U S Dep Agric 939:1–18

Olsson PA (1999) Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiol Ecol 29:303–310. https://doi.org/10.1111/j.1574-6941.1999.tb00621.x

Ooms J (2020) writexl: export data frames to excel “xlsx” format. Version 1.3.1URL https://CRAN.R-project.org/package=writexl

Opala PA, Okalebo JR, Othieno CO, Kisinyo P (2010) Effect of organic and inorganic phosphorus sources on maize yields in an acid soil in western Kenya. Nutr Cycl Agroecosystems 86:317–329. https://doi.org/10.1007/s10705-009-9294-3

Ortega JSE, Vásquez NNA, Alba TÁ, Salamone IEG de (2021) Impact of management of cover crop–soybean agroecosystems on rhizosphere microbial communities. Eur J Soil Sci 72:1154–1176. https://doi.org/10.1111/ejss.13057

Paauw F van der (1971) An effective water extraction method for the determination of plant-available soil phosphorus. Plant Soil 34:467–481. https://doi.org/10.1007/BF01372799

Pang J, Ruchi B, Zhao H, et al (2018a) The carboxylate-releasing phosphorus-mobilising strategy can be proxied by foliar manganese concentration in a large set of chickpea germplasm under low phosphorus supply. New Phytol 219:518–529

Pang J, Zhao H, Bansal R, et al (2018b) Leaf transpiration plays a role in phosphorus acquisition among a large set of chickpea genotypes. Plant Cell Environ 1–11. https://doi.org/10.1111/pce.13139

Parihar CM, Jat SL, Singh AK, et al (2016) Conservation agriculture in irrigated intensive maize-based systems of north-western India: Effects on crop yields, water productivity and economic profitability. Field Crops Res 193:104–116. https://doi.org/10.1016/j.fcr.2016.03.013

Parray JA, Jan S, Kamili AN, et al (2016) Current perspectives on plant growth-promoting rhizobacteria. J Plant Growth Regul 35:877–902. https://doi.org/10.1007/s00344-016-9583-4

Pavinato PS, Rodrigues M, Soltangheisi A, et al (2017) Effects of cover crops and phosphorus sources on maize yield, phosphorus uptake, and phosphorus use efficiency. Agron J 109:1–9. https://doi.org/10.2134/agronj2016.06.0323

Pearse SJ, Veneklaas EJ, Cawthray G, et al (2006) Triticum aestivum shows a greater biomass response to a supply of aluminium phosphate than *Lupinus albus*, despite releasing fewer carboxylates into the rhizosphere. New Phytol 169:515–524. https://doi.org/10.1111/j.1469-8137.2005.01614.x

Pedersen IF, Christensen JT, Sørensen P, et al (2021) Early plant height: A defining factor for yields of silage maize with contrasting phosphorus supply. Soil Use Manag Early View: https://doi.org/10.1111/sum.12697

Peixoto DS, Silva L de CM da, Melo LBB de, et al (2020) Occasional tillage in no-tillage systems: A global meta-analysis. Sci Total Environ 745:140887. https://doi.org/10.1016/j.scitotenv.2020.140887

Perucci P, Scarponi L (1985) Effect of different treatments with crop residues on soil phosphatase activity. Biol Fertil Soils 1:111–115. https://doi.org/10.1007/BF00255138

Philippot L, Bru D, Saby NPA, et al (2009) Spatial patterns of bacterial taxa in nature reflect ecological traits of deep branches of the 16S rRNA bacterial tree. Environ Microbiol 11:3096–3104. https://doi.org/10.1111/j.1462-2920.2009.02014.x

Piepho HP, Buchse A, Emrich K (2003) A hitchhiker's guide to mixed models for randomized experiments. J Agron Crop Sci 189:310–322. https://doi.org/10.1046/j.1439-037X.2003.00049.x

Pierre W, Parker F (1927) Soil phosphorus studies: II. The concentration of organic and inorganic phosphorus in the soil solution and soil extracts and the availability of organic phosphorus of plants. Soil Sci 24:119–128

Plenchette C, Clermont-Dauphin C, Meynard JM, Fortin JA (2005) Managing arbuscular mycorrhizal fungi in cropping systems. Can J Plant Sci 85:31–40

Poeplau C, Don A (2015) Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. Agric Ecosyst Environ 200:33–41. https://doi.org/10.1016/j.agee.2014.10.024

Poll C, Ingwersen J, Stemmer M, et al (2006) Mechanisms of solute transport affect small-scale abundance and function of soil microorganisms in the detritusphere. Eur J Soil Sci 57:583–595

Possinger AR, Byrne LB, Breen NE (2013) Effect of buckwheat (*Fagopyrum esculentum*) on soil-phosphorus availability and organic acids. J Plant Nutr Soil Sci 176:16–18. https://doi.org/10.1002/jpln.201200337

Pypers P, Huybrighs M, Diels J, et al (2007) Does the enhanced P acquisition by maize following legumes in a rotation result from improved soil P availability? Soil Biol Biochem 39:2555–2566. https://doi.org/10.1016/j.soilbio.2007.04.026

Quiquampoix H, Mousain D (2005) Enzymatic hydrolysis of organic phosphorus. In: Turner BL, Frossard E, Baldwin DS (eds) Organic phosphorus in the environment. CABI, Wallingford, pp 89–112

Ragot SA, Kertesz MA, Mészáros É, et al (2017) Soil *phoD* and *phoX* alkaline phosphatase gene diversity responds to multiple environmental factors. FEMS Microbiol Ecol 93:. https://doi.org/10.1093/femsec/fiw212

Ramos-Zapata JA, Marrufo-Zapata D, Guadarrama P, et al (2012) Impact of weed control on arbuscular mycorrhizal fungi in a tropical agroecosystem: a long-term experiment. Mycorrhiza 22:653–661. https://doi.org/10.1007/s00572-012-0443-1

Randhawa PS, Condron LM, Di HJ, et al (2005) Effect of green manure addition on soil organic phosphorus mineralisation. Nutr Cycl Agroecosystems 73:181–189. https://doi.org/10.1007/s10705-005-0593-z

Rao MA, Violante A, Gianfreda L (2000) Interaction of acid phosphatase with clays, organic molecules and organo-mineral complexes: kinetics and stability. Soil Biol Biochem 32:1007–1014. https://doi.org/10.1016/S0038-0717(00)00010-9

Ratnadass A, Fernandes P, Avelino J, Habib R (2012) Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: A review. Agron Sustain Dev 32:273–303. https://doi.org/10.1007/s13593-011-0022-4

R-Core Team (2013) R: A language and environment for statistical computing. Version 3.5.3. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Reed MS (2008) Stakeholder participation for environmental management: A literature review. Biol Conserv 141:2417–2431. https://doi.org/10.1016/j.biocon.2008.07.014

Regan K, Nunan N, Boeddinghaus R, et al (2014) Seasonal controls on grassland microbial biogeography: Are they governed by plants, abiotic properties or both? 71:21–30

Regierungspräsidium Freiburg, Landesamt für Geologie, Rohstoffe und Bergbau (2020) LGRB-Kartenviewer - Layer GeoLa-BK50:Bodenkundliche Einheiten

Rengel Z (2008) Bioavailability of phosphorus and micronutrients in the soil-plant-microbe continuum. Rev Cienc Suelo Nutr Veg 8:84–91. https://doi.org/10.4067/S0718-27912008000400013

Richardson AE, Lynch JP, Ryan PR, et al (2011) Plant and microbial strategies to improve the phosphorus efficiency of agriculture. Plant Soil 349:121–156. https://doi.org/10.1007/s11104-011-0950-4

Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability update on microbial phosphorus. PLANT Physiol 156:989–996. https://doi.org/10.1104/pp.111.175448

Rick TL, Jones CA, Engel RE, Miller PR (2011) Green manure and phosphate rock effects on phosphorus availability in a northern Great Plains dryland organic cropping system. Org Agric 1:81–90. https://doi.org/10.1007/s13165-011-0007-2

Ritz K (1995) Growth responses of some soil fungi to spatially heterogeneous nutrients. FEMS Microbiol Ecol 16:269–279. https://doi.org/10.1111/j.1574-6941.1995.tb00291.x

Ro S, Becker M, Manske G (2016) Effect of phosphorus management in rice–mungbean rotations on sandy soils of Cambodia. J Plant Nutr Soil Sci 179:481–487. https://doi.org/10.1002/jpln.201600043

Roarty S, Hackett RA, Schmidt O (2017) Earthworm populations in twelve cover crop and weed management combinations. Appl Soil Ecol 114:142–151. https://doi.org/10.1016/j.apsoil.2017.02.001

Roelofs RFR, Rengel Z, Cawthray GR, et al (2008) Exudation of carboxylates in Australian Proteaceae: chemical composition. Plant Cell Environ 24:891–904. https://doi.org/10.1046/j.1365-3040.2001.00741.x

Romanyà J, Blanco-Moreno JM, Sans FX (2017) Phosphorus mobilization in low-P arable soils may involve soil organic C depletion. Soil Biol Biochem 113:250–259. https://doi.org/10.1016/j.soilbio.2017.06.015

Romanyà J, Rovira P (2009) Organic and inorganic P reserves in rain-fed and irrigated calcareous soils under long-term organic and conventional agriculture. Geoderma 151:378–386. https://doi.org/10.1016/j.geoderma.2009.05.009

Romdhane S, Spor A, Busset H, et al (2019) Cover crop management practices rather than composition of cover crop mixtures affect bacterial communities in no-till agroecosystems. Front Microbiol 10:1–11. https://doi.org/10.3389/fmicb.2019.01618

Rose TJ, Damon P, Rengel Z (2010a) Phosphorus-efficient faba bean (*Vicia faba* L.) genotypes enhance subsequent wheat crop growth in an acid and an alkaline soil. Crop Pasture Sci 61:1009–1016

Rose TJ, Hardiputra B, Rengel Z (2010b) Wheat, canola and grain legume access to soil phosphorus fractions differs in soils with contrasting phosphorus dynamics. Plant Soil 326:159–170. https://doi.org/10.1007/s11104-009-9990-4

Rose TJ, Zwieten L van, Claassens A, et al (2018) Phytotoxicity of soilborne glyphosate residues is influenced by the method of phosphorus fertiliser application. Plant Soil 422:455–465. https://doi.org/10.1007/s11104-017-3482-8

Rowe DE, Fairbrother TE, Sistani KA (2006) Winter cover crop and management effects on summer and annual nutrient yields. Agron J 98:946–950. https://doi.org/10.2134/agronj2005.0178

RStudio (2013) RStudio: Integrated development environment for R. Version 0.97.551. Boston, MA. URL http://www.rstudio.org/

Rückamp D (2011) Role of termites for the distribution patterns of carbon and phosphorus fractions and the genesis of tropical soils, Brazil. PhD-Thesis, University of Bonn

Ruess L, Chamberlain PM (2010) The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biol Biochem 42:1898–1910. https://doi.org/10.1016/j.soilbio.2010.07.020

Rutunga V, Karanja NK, Gachene CK (2008) Six month-duration *Tephrosia vogelii* Hook. f. and *Tithonia diversifolia* (Hemsl.) A. Gray planted-fallows for improving maize production in Kenya. Biotechnol Agron Société Environ 12:267–278

Ryan MH, Graham JH (2018) Little evidence that farmers should consider abundance or diversity of arbuscular mycorrhizal fungi when managing crops. New Phytol 1–13. https://doi.org/10.1111/nph.15308

Sarrantonio M, Gallandt E (2003) The role of cover crops in North American cropping systems. J Crop Prod 8:53–74. https://doi.org/10.1300/J144v08n01_04

Sasse J, Martinoia E, Northen T (2018) Feed your friends: do plant exudates shape the root microbiome? Trends Plant Sci 23:25–41. https://doi.org/10.1016/j.tplants.2017.09.003

Schauberger P, Walker A (2019) openxlsx: read, write and edit xlsx files. Version 4.1.3URL https://CRAN.R-project.org/package=openxlsx

Schelfhout S, De Schrijver A, Verheyen K, et al (2018) Phosphorus mining efficiency declines with decreasing soil P concentration and varies across crop species. Int J Phytoremediation 20:939–946. https://doi.org/10.1080/15226514.2018.1448363

Scheu S (1992) Automated measurement of the respiratory response of soil microcompartments: Active microbial biomass in earthworm faeces. Soil Biol Biochem 24:1113–1118. https://doi.org/10.1016/0038-0717(92)90061-2

Schilling G, Gransee A, Deuhel A, et al (1998) Phosphorus availability, root exudates, and microbial activity in the rhizosphere. Z Für Pflanzenernähr Bodenkd 161:465–478. https://doi.org/10.1002/jpln.1998.3581610413

Schinner F, Öhlinger R, Kandeler E, Margesin R (eds) (1993) Bodenbiologische Arbeitsmethoden, 2nd edn. Springer-Verlag, Berlin Heidelberg

Schipanski ME, Barbercheck M, Douglas MR, et al (2014) A framework for evaluating ecosystem services provided by cover crops in agroecosystems. Agric Syst 125:12–22. https://doi.org/10.1016/j.agsy.2013.11.004

Schmidt R, Gravuer K, Bossange AV, et al (2018) Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. PLOS ONE 13:e0192953. https://doi.org/10.1371/journal.pone.0192953

Schnug E, De Kok LJ (eds) (2016) Phosphorus in agriculture: 100 % zero. Springer, Dordrecht

Schoumans OF, Chardon WJ, Bechmann ME, et al (2014) Mitigation options to reduce phosphorus losses from the agricultural sector and improve surface water quality: A review. Sci Total Environ 468–469:1255–1266. https://doi.org/10.1016/j.scitotenv.2013.08.061

Schröder JJ, Smit AL, Cordell D, Rosemarin A (2011) Improved phosphorus use efficiency in agriculture: A key requirement for its sustainable use. Chemosphere 84:822–831. https://doi.org/10.1016/j.chemosphere.2011.01.065

Schüller H (1969) Die CAL-Methode, eine neue Methode zur Bestimmung des pflanzenverfügbaren Phosphates in Böden. Z Für Pflanzenernähr Bodenkd 123:48–63. https://doi.org/10.1002/jpln.19691230106

Schützenmeister A, Jensen U, Piepho H-P (2012) Checking normality and homoscedasticity in the general linear model using diagnostic plots. Commun Stat - Simul Comput 41:141–154. https://doi.org/10.1080/03610918.2011.582560

Scopel E, Triomphe B, Affholder F, et al (2013) Conservation agriculture cropping systems in temperate and tropical conditions, performances and impacts. A review. Agron Sustain Dev 33:113–130. https://doi.org/10.1007/s13593-012-0106-9

Sharpley A (2016) Managing agricultural phosphorus to minimize water quality impacts. Sci Agric 73:1–8. https://doi.org/10.1590/0103-9016-2015-0107

Sharpley AN (1995) Soil phosphorus dynamics: agronomic and environmental impacts. Ecol Eng 5:261–279. https://doi.org/10.1016/0925-8574(95)00027-5

Sharpley AN, Smith JR (1991) Effects of cover crops on surface water quality. In: Hargrove WL (ed) West Tennessee Experiment Station April 9-11, 1991. Soil and Water Conservation Society, Jackson, Tennessee, USA

Sharrock RA, Sinclair FL, Gliddon C, et al (2004) A global assessment using PCR techniques of mycorrhizal fungal populations colonising *Tithonia diversifolia*. Mycorrhiza 14:103–109. https://doi.org/10.1007/s00572-003-0243-8

Sileshi G, Akinnifesi FK, Ajayi OC, Place F (2008) Meta-analysis of maize yield response to woody and herbaceous legumes in sub-Saharan Africa. Plant Soil 307:1–19. https://doi.org/10.1007/s11104-008-9547-y

Simpson RJ, Oberson A, Culvenor RA, et al (2011) Strategies and agronomic interventions to improve the phosphorus-use efficiency of farming systems. Plant Soil 349:89–120. https://doi.org/10.1007/s11104-011-0880-1

Six J, Frey SD, Thiet RK, Batten KM (2006) Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci Soc Am J 70:555–569. https://doi.org/10.2136/sssaj2004.0347

Smeck NE (1985) Phosphorus dynamics in soils and landscapes. Geoderma 36:185–199. https://doi.org/10.1016/0016-7061(85)90001-1

Smith A, Fressoli M, Thomas H (2014) Grassroots innovation movements: challenges and contributions. J Clean Prod 63:114–124. https://doi.org/10.1016/j.jclepro.2012.12.025

Smith SE, Anderson IC, Smith F (2015) Mycorrhizal associations and phosphorus acquisition: From cells to ecosystems. Annu Plant Rev Phosphorus Metab Plants 48:409–439. https://doi.org/10.1002/9781118958841.ch14

Smith SE, Jakobsen I, Gronlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in

arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol 156:1050–1057. https://doi.org/10.1104/pp.111.174581

Soltangheisi A, Teles APB, Sartor LR, Pavinato PS (2020) Cover cropping may alter legacy phosphorus dynamics under long-term fertilizer addition. Front Environ Sci 8:. https://doi.org/10.3389/fenvs.2020.00013

Somado EA, Becker M, Kuehne RF, et al (2003) Combined effects of legumes with rock phosphorus on rice in West Africa. Agron J 95:1172–1178. https://doi.org/10.2134/agronj2003.1172

Sorensen JN, Larsen J, Jakobsen I (2005) Mycorrhiza formation and nutrient concentration in leeks (*Allium porrum*) in relation to previous crop and cover crop management on high P soils. Plant Soil 273:101–114. https://doi.org/10.1007/s11104-004-6960-8

Spohn M, Kuzyakov Y (2013a) Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation – Coupling soil zymography with ^{14}C imaging. Soil Biol Biochem 67:106–113. https://doi.org/10.1016/j.soilbio.2013.08.015

Spohn M, Kuzyakov Y (2013b) Phosphorus mineralization can be driven by microbial need for carbon. Soil Biol Biochem 61:69–75. https://doi.org/10.1016/j.soilbio.2013.02.013

Staddon PL, Ramsey CB, Ostle N, et al (2003) Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of ^{14}C. Science 300:1138–1140. https://doi.org/10.1126/science.1084269

Steffens D, Leppin T, Luschin-Ebengreuth N, et al (2010) Organic soil phosphorus considerably contributes to plant nutrition but is neglected by routine soil-testing methods. J Plant Nutr Soil Sci 173:765–771. https://doi.org/10.1002/jpln.201000079

Stewart J, Tiessen H (1987) Dynamics of soil organic phosphorus. Biogeochemistry 4:41–60

Stutter MI, Shand CA, George TS, et al (2015) Land use and soil factors affecting accumulation of phosphorus species in temperate soils. Geoderma 257–258:29–39. https://doi.org/10.1016/j.geoderma.2015.03.020

Stutter MI, Shand CA, George TS, et al (2012) Recovering phosphorus from soil: a root solution? Environ Sci Technol 46:1977–1978. https://doi.org/10.1021/es2044745

Sugito T, Yoshida K, Takebe M, et al (2010) Soil microbial biomass phosphorus as an indicator of phosphorus availability in a Gleyic Andosol. Soil Sci Plant Nutr 56:390–398. https://doi.org/10.1111/j.1747-0765.2010.00483.x

Tadano T, Sakai H (1991) Secretion of acid phosphatase by the roots of several crop species under phosphorus-deficient conditions. Soil Sci Plant Nutr 37:129–140. https://doi.org/10.1080/00380768.1991.10415018

Takeda M, Nakamoto T, Miyazawa K, et al (2009a) Phosphorus availability and soil biological activity in an Andosol under compost application and winter cover cropping. Appl Soil Ecol 42:86–95. https://doi.org/10.1016/j.apsoil.2009.02.003

Takeda M, Nakamoto T, Miyazawa K, Murayama T (2009b) Phosphorus transformation in a soybean-cropping system in Andosol: effects of winter cover cropping and compost application. Nutr Cycl Agroecosystems 85:287–297. https://doi.org/10.1007/s10705-009-9267-6

Talgre L, Lauringson E, Roostalu H, et al (2012) Green manure as a nutrient source for succeeding crops. Plant Soil Env 58:275–281

Tanwar SPS, Rao SS, Regar PL, et al (2014) Improving water and land use efficiency of fallow-wheat system in shallow Lithic Calciorthid soils of arid region: Introduction of bed planting and rainy season sorghum–legume intercropping. Soil Tillage Res 138:44–55. https://doi.org/10.1016/j.still.2013.12.005

Tarafdar JC, Jungk A (1987) Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol Fertil Soils 3:199–204

Tarawali G, Manyong VM, Carsky RJ, et al (1999) Adoption of improved fallows in West Africa: Lessons from mucuna and stylo case studies. Agrofor Syst 47:93–122

Tarui A, Matsumura A, Asakura S, et al (2013) Evaluation of mixed cropping of oat and hairy vetch as green manure for succeeding corn production. Plant Prod Sci 16:383–392. https://doi.org/10.1626/pps.16.383

Teboh JM, Franzen DW (2011) Buckwheat (*Fagopyrum esculentum* Moench) potential to contribute solubilized soil phosphorus to subsequent crops. Commun Soil Sci Plant Anal 42:1544–1550. https://doi.org/10.1080/00103624.2011.581724

Teles APB, Rodrigues M, Bejarano Herrera WF, et al (2017) Do cover crops change the lability of phosphorus in a clayey subtropical soil under different phosphate fertilizers? Soil Use Manag 33:34–44. https://doi.org/10.1111/sum.12327

Thomson BD, Robson AD, Abbott LK (1986) Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. New Phytol 103:751–765. https://doi.org/10.1111/j.1469-8137.1986.tb00850.x

Tiecher T, dos Santos DR, Calegari A (2012a) Soil organic phosphorus forms under different soil management systems and winter crops, in a long term experiment. Soil Tillage Res 124:57–67. https://doi.org/10.1016/j.still.2012.05.001

Tiecher T, dos Santos DR, Kaminski J, Calegari A (2012b) Forms of inorganic phosphorus in soil under different long term soil tillage systems and winter crops. Rev Bras Ciênc Solo 36:271–282. https://doi.org/10.1590/S0100-06832012000100028

Tonitto C, David MB, Drinkwater LE (2006) Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: A meta-analysis of crop yield and N dynamics. Agric Ecosyst Environ 112:58–72. https://doi.org/10.1016/j.agee.2005.07.003

Tóth G, Guicharnaud R-A, Tóth B, Hermann T (2014) Phosphorus levels in croplands of the European Union with implications for P fertilizer use. Eur J Agron 55:42–52. https://doi.org/10.1016/j.eja.2013.12.008

Trappe JM, Molina R, Castellano M (1984) Reactions of mycorrhizal fungi and mycorrhiza formation to pesticides. Annu Rev Phytopathol 22:331–359

Tummers B (2006) DataThief III. Version 1.7URL http://www.datathief.org/

Turmel MS, Entz MH, Tenuta M, et al (2011) The influence of a long-term black medic (*Medicago lupulina* cv. George) cover crop on arbuscular mycorrhizal fungal colonization and nutrient uptake in flax (*Linum usitatissimum*) under zero-tillage management. Can J Plant Sci 91:1071–1076. https://doi.org/10.4141/cjps10115

Turner BL (2007) Inositol phosphates in soil: amounts, forms and significance of the phosphorylated inositol stereoisomers. In: Turner BL, Richardson AE, Mullaney EJ (eds) Inositol phosphates: Linking agriculture and the environment. CABI, Wallingford, pp 186–207

Turner BL (2008) Resource partitioning for soil phosphorus: a hypothesis. J Ecol 96:698–702. https://doi.org/10.1111/j.1365-2745.2008.01384.x

Turner BL, Cade-Menun BJ, Condron LM, Newman S (2005) Extraction of soil organic phosphorus. Talanta 66:294–306. https://doi.org/10.1016/j.talanta.2004.11.012

Turner BL, Haygarth PM (2005) Phosphatase activity in temperate pasture soils: Potential regulation of labile organic phosphorus turnover by phosphodiesterase activity. Sci Total Environ 344:27–36. https://doi.org/10.1016/j.scitotenv.2005.02.003

Turner BL, Papházy MJ, Philip M. Haygarth, Ian D. McKelvie (2002) Inositol phosphates in the environment. Philos Trans Biol Sci 357:449–469

Turner BL, Richardson AE, Mullaney EJ (eds) (2007) Inositol phosphates: linking agriculture and the environment. CABI, Wallingford

Turrini A, Sbrana C, Avio L, et al (2016) Changes in the composition of native root arbuscular mycorrhizal fungal communities during a short-term cover crop-maize succession. Biol Fertil Soils 52:643–653. https://doi.org/10.1007/s00374-016-1106-8

Uksa M, Schloter M, Endesfelder D, et al (2015) Prokaryotes in subsoil—evidence for a strong spatial separation of different phyla by analysing co-occurrence networks. Front Microbiol 6:1–13. https://doi.org/10.3389/fmicb.2015.01269

Vanden Nest T, Vandecasteele B, Ruysschaert G, Merckx R (2014) Incorporation of catch crop residues does not increase phosphorus leaching: a soil column experiment in unsaturated conditions. Soil Use Manag 30:351–360. https://doi.org/10.1111/sum.12131

Vanlauwe B, Diels J, Sanginga N, et al (2000) Utilization of rock phosphate by crops on a representative toposequence in the Northern Guinea savanna zone of Nigeria: response by maize to previous herbaceous legume cropping and rock phosphate treatments. Soil Biol Biochem 32:2079–2090. https://doi.org/10.1016/S0038-0717(00)00150-4

Vanzolini JI, Galantini JA, Martínez JM, Suñer L (2017) Changes in soil pH and phosphorus availability during decomposition of cover crop residues. Arch Agron Soil Sci 63:1864–1874. https://doi.org/10.1080/03650340.2017.1308493

Varela MF, Barraco M, Gili A, et al (2017) Biomass decomposition and phosphorus release from residues of cover crops under no-tillage. Agron J 109:317–326. https://doi.org/10.2134/agronj2016.03.0168

Vazquez C, de Goede RGM, Rutgers M, et al (2021) Assessing multifunctionality of agricultural soils: Reducing the biodiversity trade-off. Eur J Soil Sci 72:1624–1639. https://doi.org/10.1111/ejss.13019

VDLUFA (1995a) Methode A 3.5.2.7: Bestimmung von Gesamt-Stickstoff: Verbrennungsmethode. In: Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik. Band II.1 Die Untersuchung von Düngemitteln, 4th edn. VDLUFA Verlag, Darmstadt

VDLUFA (1995b) Methode A 8.10: Bestimmung von Mikronährstoffen in Düngemittelextrakten, ICP-OES-Methode. In: Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik. Band II.1 Die Untersuchung von Düngemitteln, 4th edn. VDLUFA Verlag, Darmstadt

VDLUFA (2012) Methode A 6.2.1.1: Bestimmung von Phosphor und Kalium im Calcium-Acetat-Lactat-Auszug. In: Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik. Band I Die Untersuchung von Böden, 6th edn. VDLUFA Verlag, Darmstadt

Veneklaas EJ, Stevens J, Cawthray GR, et al (2003) Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. Plant Soil 248:187–197. https://doi.org/10.1023/A:1022367312851

Vetterlein D, Carminati A, Kögel-Knabner I, et al (2020) Rhizosphere spatiotemporal organization–a key to rhizosphere functions. Front Agron 2:8. https://doi.org/10.3389/fagro.2020.00008

Villamil MB, Bollero GA, Darmody RG, et al (2006) No-till corn/soybean systems including winter cover crops. Soil Sci Soc Am J 70:1936–1944. https://doi.org/10.2136/sssaj2005.0350

Vos HMJ, Ros MBH, Koopmans GF, van Groenigen JW (2014) Do earthworms affect phosphorus availability to grass? A pot experiment. Soil Biol Biochem 79:34–42. https://doi.org/10.1016/j.soilbio.2014.08.018

Wang B, Qiu Y-L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363. https://doi.org/10.1007/s00572-005-0033-6

Wang G, Ngouajio M, Warncke DD (2008) Nutrient cycling, weed suppression, and onion yield following brassica and sorghum sudangrass cover crops. HortTechnology 18:68–74

Wang Y, Krogstad T, Clarke JL, et al (2016) Rhizosphere organic anions play a minor role in improving crop species' ability to take up residual phosphorus (P) in agricultural soils low in p availability. Front Plant Sci 7:1–14. https://doi.org/10.3389/fpls.2016.01664

Weerakoon WL, Seneviratne G, Silva MAPD, Seneviratine AM (1992) Evaluation of *Sesbania speciosa* as a green manure for lowland rice in the dry zone, Sri Lanka. Plant Soil 145:131–139. https://doi.org/10.1007/BF00009549

Weil R, Kremen A (2007) Thinking across and beyond disciplines to make cover crops pay. J Sci Food Agric 87:551–557. https://doi.org/10.1002/jsfa.2742

Weil RR (2000) Soil and plant influences on crop response to two African phosphate rocks. Agron J 92:1167–1175. https://doi.org/10.2134/agronj2000.9261167x

Weisskopf L, Abou-Mansour E, Fromin N, et al (2006) White lupin has developed a complex strategy to limit microbial degradation of secreted citrate required for phosphate acquisition. Plant Cell Environ 29:919–927. https://doi.org/10.1111/j.1365-3040.2005.01473.x

Weller DM, Raaijmakers JM, Gardener BBM, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348. https://doi.org/10.1146/annurev.phyto.40.030402.110010

Wendling M, Büchi L, Amossé C, et al (2016) Influence of root and leaf traits on the uptake of nutrients in cover crops. Plant Soil 409:419–434. https://doi.org/10.1007/s11104-016-2974-2

Wezel A, Herren BG, Kerr RB, et al (2020) Agroecological principles and elements and their implications for transitioning to sustainable food systems. A review. Agron Sustain Dev 40:40. https://doi.org/10.1007/s13593-020-00646-z

White CM, Weil RR (2010) Forage radish and cereal rye cover crop effects on mycorrhizal fungus colonization of maize roots. Plant Soil 328:507–521. https://doi.org/10.1007/s11104-009-0131-x

White CM, Weil RR (2011) Forage radish cover crops increase soil test phosphorus surrounding radish taproot holes. Soil Sci Soc Am J 75:121–130. https://doi.org/10.2136/sssaj2010.0095

White DC, Davis WM, Nickels JS, et al (1979) Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40:51–62. https://doi.org/10.1007/BF00388810

Wickham H (2009) ggplot2: elegant graphics for data analysis. Version 3.2.1. Springer, New York. URL https://cran.r-project.org/web/packages/ggplot2/index.html

Wickham H (2011) The Split-Apply-Combine strategy for data analysis. J Stat Softw 40:1–29

Wickham H, Averick M, Bryan J, et al (2019a) Welcome to the tidyverse. J Open Source Softw 4:1686. https://doi.org/10.21105/joss.01686

Wickham H, Bryan J (2018) readxl: read excel files. Version 1.3.1URL https://CRAN.R-project.org/package=readxl

Wickham H, François R, Henry L, Müller K (2019b) dplyr: a grammar of data manipulation. Version 1.8.6URL https://CRAN.R-project.org/package=dplyr

Wilke CO (2017) cowplot: streamlined plot theme and plot annotations for “ggplot2.” Version 0.9.2URL https://CRAN.R-project.org/package=cowplot

Willers C, Jansen van Rensburg PJ, Claassens S (2015) Phospholipid fatty acid profiling of microbial communities-a review of interpretations and recent applications. J Appl Microbiol 119:1207–1218. https://doi.org/10.1111/jam.12902

Winiwarter V (2002) Landwirtschaftliches Wissen vom Boden. Zur Geschichte der Konzepte eines praktischen Umgangs mit der Erde. In: Kunst- und Ausstellungshalle der Bundesrepublik Deutschland. Wiss. Red. Bernd Busch, Erde, Wienand, Köln

Withers PJA, Doody DG, Sylvester-Bradley R (2018) Achieving sustainable phosphorus use in food systems through circularisation. Sustainability 10:1804. https://doi.org/10.3390/su10061804

Wittwer RA, Dorn B, Jossi W, Heijden MGA van der (2017) Cover crops support ecological intensification of arable cropping systems. Sci Rep 7:1–12. https://doi.org/10.1038/srep41911

Wortman SE, Drijber RA, Francis CA, Lindquist JL (2013) Arable weeds, cover crops, and tillage drive soil microbial community composition in organic cropping systems. Appl Soil Ecol 72:232–241. https://doi.org/10.1016/j.apsoil.2013.07.014

Wyss M, Brugger R, Kronenberger A, et al (1999) Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): catalytic properties. Appl Env Microbiol 65:367–373

Xie J, Schoenau J, Warkentin TD (2017) Yield and uptake of nitrogen and phosphorus in soybean, pea, and lentil and effects on soil nutrient supply and crop yield in the succeeding year in Saskatchewan, Canada. Can J Plant Sci. https://doi.org/10.1139/cjps-2016-0342

Yamada T, Kremer RJ, de Camargo e Castro PR, Wood BW (2009) Glyphosate interactions with physiology, nutrition, and diseases of plants: Threat to agricultural sustainability? Eur J Agron 31:111–113. https://doi.org/10.1016/j.eja.2009.07.004

Young IM, Ritz K (2000) Tillage, habitat space and function of soil microbes. Soil Tillage Res 53:201–213. https://doi.org/10.1016/S0167-1987(99)00106-3

Zhu H (2021) kableExtra: construct complex table with “kable” and pipe syntax. Version 1.5.3URL https://CRAN.R-project.org/package=kableExtra

Zhu Y, Yan F, Zörb C, Schubert S (2005) A link between citrate and proton release by proteoid roots of white lupin (*Lupinus albus* L.) grown under phosphorus-deficient conditions? Plant Cell Physiol 46:892–901. https://doi.org/10.1093/pcp/pci094

Zibilske LM, Makus DJ (2009) Black oat cover crop management effects on soil temperature and biological properties on a Mollisol in Texas, USA. Geoderma 149:379–385. https://doi.org/10.1016/j.geoderma.2009.01.001

Zikeli S, Gruber S (2017) Reduced tillage and no-till in organic farming systems, Germany—status quo, potentials and challenges. Agriculture 7:35. https://doi.org/10.3390/agriculture7040035

Zuo Y, Zhang F (2008) Effect of peanut mixed cropping with gramineous species on micronutrient concentrations and iron chlorosis of peanut plants grown in a calcareous soil. Plant Soil 306:23–36. https://doi.org/10.1007/s11104-007-9484-1

Zuo Y, Zhang F, Li X, Cao Y (2000) Studies on the improvement in iron nutrition of peanut by intercropping with maize on a calcareous soil. Plant Soil 220:13–25. https://doi.org/10.1023/A:1004724219988

Declaration

Declaration in lieu of an oath on independent work according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

1. The dissertation submitted on the topic

Soil microorganisms as hidden miners of phosphorus in soils under different cover crop and tillage

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

Girona, December 12th 2021 Moritz Hallama

Curriculum Vitae

(updated December 10th 2021)

Personal data

Name	Moritz Hallama
Date of birth	September 28th 1984
Civil state	Married
Children	Aran (2008) and Uba (2010)
Nationality	German
NIE	X9251948Z
Address	Neershofer Strasse 152, D-96450 Coburg
E-mail	moeha@gmx.net / moritz.hallama@uni-hohenhim.de
Phone	(+49) 17634739915
ORCID	0000-0003-4209-6760
Researchgate	https://www.researchgate.net/profile/Moritz-Hallama
Current position	PhD-student (University of Hohenheim, Germany), Associate Lecturer at UdG (Girona, Catalonia, Spain)

Academic qualifications

09/2015-ongoing	**PhD-Thesis** "Optimization of Phosphorus Dynamics using Cover Crop Management and Reduced Tillage" Tutors: Prof Ellen Kandeler and Prof Carola Pekrun. Department of Soil Biology, University of Hohenheim, Germany
10/2010 – 05/2014:	**MSc**: **Environmental protection and Agricultural Food Production**. Grade: A (1.1) MSc-Thesis (Department of Soil Biology): Mulching with Silage Changes Function and Abundance of Soil Microbial Communities and Affects Late Blight in Organic Potatoes**.** Grade: A Universität Hohenheim, Germany
09/2005 – 02/2010:	Llicenciatura (Bachelor) en Ciencies Ambientals (Environmental

	Sciences). Specialization: Natural sciences/ technology and Management/Planning. Grade: B Final paper: *Greenwashing - The Impact of "green marketing" on Spanish Consumers* (El Fenomen del Greenwashing. Avaluació de l'Impacte a l'Estat Espanyol). Grade: B Universitat de Girona, Spain
11/2003 - 02/2005	**Civil Service**, WI - Weltweite Initiative für Soziales Engagement, Masaya, **Nicaragua.** School support and English lessons for children with risk of social exclusion. Additionally computer lessons, agricultural and kitchen work, as well as cultural/creative activities.
05/2003	Odenwaldschule, Ober-Hambach/ Heppenheim**. A-levels** (Abitur/Allgemeine Hochschulreife), **Grade: 1,8 (B+)** Main subjects: chemistry, physics Additionally: Formation as chemical-technical assistant (CTA)

Journal articles

Hallama M, Pekrun C, Mayer-Gruner P, Uksa M, et al (submitted) The role of microbes in the increase of organic phosphorus availability in the rhizosheath of cover crops, Plant Soil

Christensen JT, Hansen EM, **Hallama** M, Kandeler E, Rubæk GH (submitted) Oat, corncockle and lupine growth affects resin-extractable soil phosphorus and soil microbial properties differently, Journal of Plant Nutrition and Soil Science

Christensen JT, Hansen EM, Kandeler E, **Hallama** M, et al (2021) Effect of soil P status on barley growth, P uptake, and soil microbial properties after incorporation of cover crop shoot and root residues. Journal of Plant Nutrition and Soil Science Early View: https://doi.org/10.1002/jpln.202100046

Hallama M, Pekrun C, Pilz S, et al (2021) Interactions between cover crops and soil microorganisms increase phosphorus availability in conservation agriculture. Plant Soil. https://doi.org/10.1007/s11104-021-04897-x

Hallama M (2020) Research commentary: Biological functions in agriculture: cover crops and soil microorganisms increase the availability of organic P. IMS Newsletter 1:6–8

Hallama M, Pekrun C, Lambers H, Kandeler E (2019) Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. Plant Soil 434:7–45. https://doi.org/10.1007/s11104-018-3810-7

George TS, Giles CD, Menezes-Blackburn D, et al (2017) Organic phosphorus in the terrestrial environment: a perspective on the state of the art and future priorities. Plant Soil 1–18. doi: 10.1007/s11104-017-3391-x

Wang Y, Krogstad T, Clarke JL, **Hallama** M, et al (2016) Rhizosphere organic anions play a minor role in improving crop species' ability to take up residual phosphorus (P) in agricultural soils low in p availability. Frontiers in Plant Science 7:1664. doi: 10.3389/fpls.2016.01664

Hallama M, Montlló Ribo M, Rofas Tudela S, Ciutat Vendrell G (2011) El fenómeno del greenwashing y su impacto sobre los consumidores propuesta metodológica para su evaluación. aposta - revista de ciencias sociales 50. Retrieved from http://www.apostadigital.com/revistav3/hemeroteca/moritz.pdf

Book chapters

Crotty F, Hannula E, **Hallama** M, Kandeler E (2022) Can soil improving cropping systems reduce the loss of soil biodiversity within agricultural soils? In: Reyes-Sánchez Laura B, Horn R, Constantini EAC (eds) Sustainable soil management as a key to preserving soil biodiversity and stopping its degradation, IUSS Book in edition process

Participation in research projects

2016-2019 SoilCare - For profitable and sustainable crop production in Europe (Horizon 2020 Programme grant agreement no 677407), Total cost: 7 628 403 Euro. 28 European partners. Coordination: DLO-Alterra (Netherlands)

Conference attendance

Hallama, M (2015) "Optimization of phosphorus dynamics using cover crop management and reduced tillage", **Poster presentation**, March 17-18, 2015 Meeting of the Commission III (Soil Biology and Soil Ecology), German Soil Science Society (DBG), Bremen, Germany

Hallama, M; Jarosch, K ; Pekrun, C; Kandeler, E (2015) "Soil Microbial Phosphorus Dynamics are Affected by Cover Crops and Minimum Tillage", **Poster presentation**, Ecology of Soil Microorganisms 2015, Microbes as important drivers of soil processes, 29.11. - 3.12. 2015, Prague, Czech Republic

Hallama, M; Jarosch, K ; Pekrun, C; Kandeler, E (2015) "Optimization of Phosphorus Dynamics using Cover Crop Management and Reduced Tillage", **Poster presentation**, 58th annual meeting of the german society of soil science (DBG) 5.-10. September 2015 Munich, Germany

Hallama, M; Pekrun, C; Kandeler, E (2016) "Optimization of P Dynamics using Cover Crops and Reduced Tillage: Preliminary Results from the Tachenhausen Site, Germany" **Poster presentation**, SoilCare Project Kick-off Meeting in Leuven, Belgium

Hallama, M; Pekrun, C; Kandeler, E (2016) "Soil Microbial Phosphorus Dynamics are Affected by Cover Crops and Minimum Tillage", **Oral presentation**, Organic Phosphorus Workshop, Lake District, England, 5th - 9th September 2016

Hallama, M; Pekrun, C; Kandeler, E (2016) "Optimization of Phosphorus Dynamics using Cover Crop Management and Minimum Tillage", **Poster presentation** ,Meeting of the German Association for Plant Production GPW 27-29th September 2016 Giessen, Germany

Hallama, M; Pekrun, C; Kandeler, E (2017) "Konservierender Ackerbau unter besonderer Berücksichtigung der P-Mobilisierung durch Zwischenfrüchte" **Oral presentation,** At the event "Wege zur nachhaltigen Pflanzenproduktion - ackerbauliche Lösungen für die Zukunft ?" organized by the Working Group of Conservation Agriculture and Direct Seeding Baden-Württemberg, 19.1.2017

Hallama, M; Pekrun, C; Kandeler, E (2017) "Hidden miners: the role of microorganisms under cover crops for phosphorus dynamics" **Oral presentation,** 59th Meeting of the German Association for Soil Science 2017 Göttingen, Germany 4-9th Sept 2017

Hallama, M; Pekrun, C; Kandeler, E (2018) "Hidden miners: the role of microorganisms under cover crops for phosphorus dynamics. Results from a meta-analysis" **Oral presentation** SoilCare Plenary Meeting, Billund, Denmark, 28th May-1st June 2018

Hallama, M; Pekrun, C; Lambers, H; Kandeler, E (2018) "Hidden miners: soil-plant-microbe interactions for phosphorus mobilization with cover crops" **Oral presentation**, 6th symposium on Phosphorus in Soils and Plants, PSP6, Monday 10 September 2018 – Thursday 13 September 2018, Leuven (Belgium)

Hallama, M; Pekrun, C; Kandeler, E (2021) "Cover Crop-Microbial Interactions Increase the Availability of Organic P Pools: An Option to Tighten P Cycling" **Oral presentation**, EUROSOIL virtual congress, Geneve, Switzerland, 23rd -27th August 2021

Dissemination:

Hallama M, Pilz S, Kandeler E, Pekrun C (2020a) Zwischenfrüchte knacken den Phosphor-Safe. Ö 2:14–17

Hallama M, Pilz S, Kandeler E, Pekrun C (2020b) Bodenphosphate effizienter nutzen. Landwirtschaft ohne Pflug 5:8

Hallama M, Pilz S, Kandeler E, Pekrun C (2019) Den Phosphor-Safe knacken. BWAgrar 11:20

Review activity

(https://publons.com/researcher/2997191/moritz-hallama/)

Agriculture, ecosystems & environment (5)

European journal of soil biology (1)

Plant and soil (5)

Soil research (1)

Pedosphere (1)

Languages

- English: Fluently
- Spanish: Fluently
- Catalan: Fluently
- French: Good knowledge
- Greek: Basic knowledge
- German: Native speaker

Teaching

Year	Description	Activity	Location
2021	Curs 2020-21. 3103G02076 Pràctiques integrades de citologia i histologia, microbiologia i genètica. **Basic methodologies in cell biology, gentics and microbiology**.	Lab course in microbiology	UdG

2020	Curs 2019-20. 3103G01084 Pràctiques de microbiologia. **Basic techniques in microbiology**. Cultivation and maintenaince of bacterial strains. Sterile work and aseptic manipulation of bacterial cultures. Staining and microscopical observation of microorganisms.	Lab course	UdG
2020	Curs 2019-20. 3103G00100 Fisiologia bacteriana. **Bacterial Physiology**. The functioning of the bacterial cell. Inner and Outer cell structures, synthesis and regulation. Adaptation of the microbial cell to environemental stress factors. Methods in the analysis of bacterial physiology and genetics. Metabolic models. Regulation of the cell functions and gene expression. Signalling pathways of the bacterial cell.	Lab course	UdG
2016, 2017, 2018	**Environmental Pollution and Soil Organisms** (3102-440). MSc "Environmental protection and agricultural food production"	Lab course and lecture	University of Hohenheim
2017	Einführung in die Bodenbiologie (3102-211): Reaktion von Bodenmikroorganismen auf die Klimaerwärmung **(Introduction to Soil Biology: Reaction of Soil Microorganisms to Climate Change)**	Lecture	University of Hohenheim
2011, 2012	Tutor/Assistant in the Module "**Spatial Data Analysis with GIS**". Introduction in ESRI *ArcGIS*, a program for computer-assisted cartography and geostatistics.	Computer course	University of Hohenheim

Girona, December 10th 2021 (Moritz Hallama)