

Functional Ecology

Interactions between belowground traits and rhizosphere fungal and bacterial communities for phosphorus acquisition

Authors : Nicolas Honvault^{1,2*}, David Houben¹, Stéphane Firmin¹, Hacène Meglouli^{3,5}, Frédéric Laruelle³, Joël Fontaine³, Anissa Lounès-Hadj Sahraoui³, Arnaud Coutu⁶, Hans Lambers⁴, Michel-Pierre Faucon^{1*}

¹ AGHYLE (SFR Condorcet FR CNRS 3417), UniLaSalle, 19 Rue Pierre Waguet, 60026 Beauvais, France

² VIVESCIA 2 Rue Clément Ader, 51100 Reims, France

³ Université du Littoral Côte d'Opale, Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV, UR 4492), SFR Condorcet FR CNRS 3417, 50 rue Ferdinand Buisson, 62228 Calais cedex, France

⁴ School of Biological Sciences and Institute of Agriculture, University of Western Australia

⁵ Institut de Recherche en Biologie Végétale, Département de sciences biologiques, Université de Montréal, 4101 Sherbrooke Est, H1X 2B2, Montréal, QC, Canada

⁶ VAM2IN, UniLaSalle, 19 Rue Pierre Waguet, 60026 Beauvais, France

* Corresponding authors

Acknowledgements

The authors thank Vivescia for their financial and technical assistance. We also thank Hacène Meglouli, Erika Samain, Matthieu Forster, Céline Roisin and Aurore Coutelier for their technical assistance. The project received funding from the ANRT (Association Nationale Recherche Technologie).

Authors' Contributions

Michel-Pierre Faucon, David Houben and Anissa Lounès-Hadj Sahraoui helped define the experimental design. Hacène Meglouli, Nicolas Honvault, Frédéric Laruelle and Joël Fontaine carried out the experiment. Stéphane Firmin, Hans Lambers and Arnaud Coutu helped process and interpret the data. All authors contributed to the final version of the manuscript.

Data availability statement

Data deposited in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.rv15dv47s>, (Honvault et al. 2021).

MR NICOLAS HONVAULT (Orcid ID : 0000-0001-7855-7712)

DR HANS LAMBERS (Orcid ID : 0000-0002-4118-2272)

Article type : Research Article

Abstract

1. Plant-soil-microbe interactions play a central role in plant nutrient acquisition and thus ecosystem functioning and nutrient availability in agroecosystems. Adjustments in root morphology, root exudation and associations with microorganisms such as arbuscular mycorrhizal fungi are common for phosphorus acquisition. Yet how plant belowground functional traits interact with microbial communities for P-acquisition remains largely unknown, limiting our understanding of phosphorus availability in agroecosystems.
2. Interactions between belowground functional traits and rhizosphere soil microbial communities for P-acquisition were investigated across eight herbaceous species with contrasting root traits. Root morphological and physiological traits involved in P-acquisition were quantified simultaneously with PLFA (phospholipid fatty acid) and NLFA (neutral lipid fatty acid) microbial bioindicators.
3. Multiple correlations were observed between root morphology, root exudates and rhizosphere fungal and bacterial communities. Root exudates and in particular release of malate and malonate were strongly linked with indicators of Gram-negative bacteria, which were correlated with changes in rhizosphere soil P concentration and plant P content.
4. Our results suggest that root exudation of carboxylates may play an important role in plant-soil-microbe interactions for P-acquisition, underlining their likely role in shaping microbial communities. Incorporating these interactions in

biogeochemical models would lead to better predicting power and understanding of P cycling and ecosystem functioning.

Key words: phosphorus acquisition, plant-soil microbe interactions, PLFA, NLFA

Résumé

1. Les interactions plante-sol-microbe jouent un rôle central dans l'acquisition d'éléments nutritifs par les plantes et par conséquent dans le fonctionnement des écosystèmes et la disponibilité des nutriments dans les agroécosystèmes. L'acquisition du phosphore nécessite souvent des ajustements entre morphologie et exsudations racinaires ainsi que l'association des racines avec les micro-organismes du sol tels que les champignons mycorhiziens arbusculaires. Les mécanismes par lesquels les traits fonctionnels des plantes interagissent avec les communautés microbiennes pour l'acquisition de P restent pourtant largement méconnus, limitant ainsi notre compréhension de la disponibilité du phosphore dans les agroécosystèmes.
2. Les interactions entre traits fonctionnels souterrains et communautés microbiennes du sol rhizosphérique pour l'acquisition de P ont été étudiées au sein de huit espèces herbacées aux traits racinaires contrastés. De multiples traits morphologiques et physiologiques racinaires impliqués dans l'acquisition de P ont été quantifiés simultanément avec plusieurs bioindicateurs microbiens (PLFA et NLFA).
3. De nombreuses corrélations ont été observées entre la morphologie racinaire, les exsudats racinaires et les communautés fongiques et bactériennes du sol rhizosphérique. Les exsudats racinaires, en particulier la libération d'acide malique et malonique, étaient fortement liés aux indicateurs de bactéries Gram-négatives, eux-mêmes corrélés aux changements de la concentration en P du sol rhizosphérique et à la teneur en P des plantes.
4. Nos résultats suggèrent que l'exsudation racinaire des carboxylates joue un rôle important dans les interactions plante-sol-microbe pour l'acquisition de P, soulignant leur rôle probable dans la formation des communautés microbiennes. L'intégration de ces interactions dans les modèles biogéochimiques conduirait à une meilleure précision ainsi qu'à une meilleure compréhension du cycle du P et du fonctionnement des écosystèmes.

List of abbreviations:

AMF : Arbuscular mycorrhizal fungi

AMF NLFA : Concentration of C16:1 ω 5 neutral lipids

Gram-negative NLFA : Sum of concentrations of cy17:0, C18:1 ω 7, cy:19:0 neutral lipids

Gram-positive NLFA : Sum of concentrations of i15:0, a15:0, i16:0, i17:0, a17:0 neutral lipids

Fungi NLFA : Concentration of C18:2 ω 6,9 neutral lipid

Bacterial NLFA : Sum of Gram-negative NLFA and Gram-positive NLFA

NLFA : Neutral lipid fatty acid

N/P AMF : Ratio of concentration of C16:1 ω 5 neutral lipid and C16:1 ω 5 phospholipid

Gram-negative PLFA : Sum of concentrations of cy17:0, C18:1 ω 7, cy:19:0 phospholipid

Gram-positive PLFA : Sum of concentration of i15:0, a15:0, i16:0, i17:0, a17:0 phospholipid

Fungi PLFA : Concentration of C18:2 ω 6,9 phospholipid

Bacterial PLFA : Sum of concentration of Gram-negative PLFA and Gram-positive PLFA

PLFA : Phospholipid fatty acid

PME : Phosphomonoesterase activity in the rhizosheath

RLD : Root length density

RSA : Root surface area

SRL : Specific root length

Δ microbial P : Difference in microbial P concentration between rhizosheath and bulk soil

Δ Olsen P : Difference in Olsen P concentration between rhizosheath and bulk soil

Δ resin P : Difference in resin P concentration between rhizosheath and bulk soil

Δ pH : Difference in pH between rhizosheath and bulk soil

Introduction

Phosphorus (P) is a key limiting nutrient in most ecosystems, playing a central role in ecosystem functioning via its influence on net productivity and carbon cycling (Nottingham et al. 2015). Understanding and managing natural ecosystems and agroecosystems functioning thus requires fully grasping the factors involved in P cycling, and notably the influence of plants on P cycling via plant P-acquisition and subsequent release via decomposition. Multiple factors influence plant P-acquisition such as plant P-acquisition strategies, and in particular root traits (Wendling et al. 2016), soil microbial communities (Smith and Smith 2011; Richardson et al. 2011), climate (Hou et al. 2018) as well as soil properties. Interactions between these factors, and especially between plant P-acquisition traits and soil microorganisms are not fully understood (Legay et al. 2014; Bardgett et al. 2014; Sasse et al. 2018; Sweeney et al. 2021). Gaining a better understanding of the complex relationships between plants and soil microorganisms, as mediated by soil properties and plant functional traits is thus central to gain insight into plant P-acquisition and in turn its effects on P cycling and ecosystem functioning (Hunter et al. 2014).

A vast array of morphological, architectural, physiological and biotic traits can allow plants to acquire P either directly or indirectly through interactions with microbial communities (Campos et al. 2018). Through these traits, plants can acquire P from the diverse pools of P present in soils, from readily available inorganic P to sparingly available inorganic and organic P such as P sorbed onto clays and Al/Fe oxides or slightly soluble Al, Fe, Ca-phosphates. Spatially uneven pools of available inorganic P can be acquired via P-foraging traits such as specific root length (SRL), root length density (RLD) or root hair length and abundance (Fort 2013; Haling et al. 2018; Ma et al. 2018), allowing plants to develop an extensive surface for absorption to efficiently explore larger soil volumes (Hinsinger et al. 2018). P-mobilisation traits such as root exudation of protons, carboxylates or enzymes can also allow plants to mobilise poorly available P, that is increase soil inorganic P concentration and thus P availability by transforming P forms such as precipitates, P bound to soil particles or organic P. Precipitated inorganic P forms such as Ca/Mg-phosphate can be dissolved via proton release in the rhizosphere soil (White and Hammond 2008; Gómez-Suárez et al. 2020). Plant-released carboxylates, by competing with P for

binding sites and complexing cations (e.g., Al, Fe and Ca), can also promote P desorption from soil constituents (Wang and Lambers 2020). Finally, organic P can be taken up after its mineralization by phosphomonoesterase, phosphodiesterase or phytases released by roots (Richardson et al. 2011; Nobile et al. 2019). In addition to the direct effects of plant traits involved in P foraging and P mobilization, the activity and composition of soil microbial communities also strongly influences plant P-acquisition (Smith and Smith 2011). Association with microbial groups can indeed contribute considerably to plant P-acquisition such as extensively described for arbuscular mycorrhizal fungi (AMF) (van der Heijden et al. 2008). Several microbial groups such as phosphate solubilising bacteria, AMF or plant growth promoting rhizobacteria (PGPR) can contribute to plant P uptake via the mobilisation of organic P and unavailable inorganic P present in soils and their effect on root growth (Richardson et al. 2011; Menezes-Blackburn et al. 2018). Plant P-acquisition traits could also play an important role in shaping these bacterial and fungal communities notably via the release of root exudates (Legay et al. 2014; Bardgett 2017; Sweeney et al. 2021), jointly influencing plant P-acquisition and thus P cycling in agroecosystems. Our understanding of the role of plant traits and notably root exudates in shaping rhizosphere microbial communities for plant P-acquisition, however, remains limited (Hunter et al. 2014; Martin et al. 2016; Datta et al. 2020). Much, indeed, remains unknown on how specific belowground functional traits influence fungal and bacterial communities and the contribution of these plant-soil microorganism interactions to plant P-acquisition (Bardgett et al. 2014; Martin et al. 2016; Bardgett 2017; Sasse et al. 2018).

Several studies have underlined the considerable influence of plants on rhizosphere soil microbial communities' composition and activity, which in turn play a central role in P-acquisition (Parniske 2008; Smith and Smith 2011; Richardson and Simpson 2011). The contribution of species identity as compared to environmental conditions remains discussed (Defrenne et al. 2019; Sweeney et al. 2021). Some cover crops species such as White Lupine for example have however been observed to strongly and consistently influence microbial communities and lead to lasting effects on P availability (Marschner et al. 2002; Hallama et al. 2019), prompting more studies on the role of plant-microbe interactions in increasing P availability in agroecosystems via cover cropping. Plant functional traits such as root exudates are likely key driver

of these plant-microbe interactions, while phylogenetic patterns and effects of species identity also contribute (Barberán et al. 2015; Sweeney et al. 2021). A large diversity of plant exudates such as amino acids, organic acids, sugars, phenolic compounds and many secondary metabolites indeed play a role in shaping and communicating with soil microbial community composition and abundance (Marschner et al. 2002; Micallef et al. 2009; Harrison and Bardgett 2010; Eisenhauer et al. 2017; Sasse et al. 2018; Datta et al. 2020), and, therefore, could influence P dynamics and availability in agroecosystems. The rhizosphere microbial community is likely the result of multiple exudates playing stimulating or inhibitory roles (Hartmann et al. 2009; Datta et al. 2020). Carboxylates in particular can provide a carbon source for microbial growth, and have considerable impacts on rhizosphere microbial community composition (Marschner et al. 2002; Martin et al. 2016; Paterson et al. 2007; Adeleke et al. 2017; Hu et al. 2018), suggesting their role among the multiple overlapping mechanisms contributing to plant-microbe interactions (Hartmann et al. 2009). Changes in rhizosphere soil microbial community composition and activity due to root exudations may in turn influence P solubilization and subsequent uptake by plants (Richardson 2001). Between 1 and 50% of rhizosphere soil bacteria are indeed P-solubilising bacteria (Kalayu 2019). Root morphological traits similarly interact with microbial communities such as observed in the case of plant-growth-promoting rhizobacteria synthesizing growth regulators that can influence P-foraging traits and plant P uptake (Spaepen and Vanderleyden 2011). Plants with fine, fibrous roots are less colonized by mycorrhizal fungi (Wen et al. 2019), while this association can strongly contribute to plant P-acquisition in coarser roots (Smith and Smith 2011), likely due to a “collaboration gradient” between root traits, association with fungi and nutrient-foraging strategy (Bergmann et al. 2020). In addition, patterns between root morphological traits and root exudation have been observed (Wen et al. 2019; Honvault et al. 2020) jointly influencing microbial communities and subsequently plant P uptake. However, the role of soil microbial groups in plant P-acquisition is often studied independent of the influence of physiological and morphological root traits, and mostly focus on a single or a few bacterial or fungal species. Overall, our knowledge of the mechanisms and plant traits interacting with rhizosphere microbial community for nutrient acquisition and uptake remains limited, and further investigation is warranted. The role of carboxylate exudation for P-acquisition has been extensively described, while their

impact on rhizosphere microbial communities and feedback effect on P availability and plant P uptake has received less attention (Hunter et al. 2014; Martin et al. 2016).

In addition to our limited understanding of the role of root traits in plant-microbe interactions for P-acquisition trait plasticity also remains a major source of uncertainty when examining plant-microbe interactions for P-acquisition. Root traits have indeed been observed to present high but uneven plasticity in response to environmental conditions (Bardgett et al. 2014). Intraspecific trait variation induced by plasticity could play an important role in shaping rhizosphere microbial communities structure as well as their consequences on nutrient availability (Sweeney et al. 2021). Interactions between plant traits and soil microbial communities mediated by trait plasticity in response to environmental change were demonstrated (Li et al. 2017; Zhang et al. 2019; Defrenne et al. 2019), however our understanding of the importance of trait plasticity in response to soil properties for microbial communities and their consequences on P uptake is limited.

Moreover, due to the carbon cost associated with traits expression and microbial associations, multiple trade-offs and interactions exist between P-acquisition traits as well as their efficiency in acquiring P and rhizosphere soil microbial community composition and activity that also remain sources of uncertainty to be fully explored. Lower carboxylate-release rates have been observed in mycorrhizal roots for instance, although inconsistencies between species have also been noted (Ryan et al. 2012). The P-mobilizing efficiencies of carboxylates have moreover been proposed to be linked negatively with their metabolization rate by soil microorganisms (Wang and Lambers 2020), while increased microorganism abundance and activity prompted by exudates can also contribute to P mobilisation. This highlights the need to better understand plant and microbial mechanisms for P-acquisition and their interactions in the rhizosphere (Richardson et al. 2011). Gaining a better understanding of plant-microbe interaction for P-acquisition thus requires investigating not only the complex and likely overlapping relationships between root traits and rhizosphere microbial community, but also the importance of trait plasticity and trade-offs in these interactions (Hartmann et al. 2009; Hunter et al. 2014; Legay et al. 2014; Bardgett et al. 2014; Bardgett 2017).

The objective of this study was to examine the interactions between plant P-acquisition traits and microbial communities, and to assess their role and contribution to plant P-acquisition. In order to examine plant-soil microorganism interactions, multiple belowground functional traits of eight cover crop species of two families were measured together with fungal and bacterial bio-indicators in their rhizosphere in two soil types. The interactions between plants P-acquisition strategies and rhizosphere microbial communities were investigated via morphological and physiological traits involved in P-acquisition and their consequences for P-acquisition, as mediated by soil type. We examined trade-offs between root morphology and, in particular, fine root percentage and association with microbial communities and their consequences for P-acquisition. We expected stronger AMF indicators in the rhizosphere of coarser roots, and strong relationships between AMF indicators and P-acquisition. Interactions between carboxylate-release rates and fungal and bacterial communities were investigated to gain a better understanding of the role of carboxylate release in the complex plant-soil microorganism interactions involved in P-acquisition. Our hypothesis was that carboxylate-release would influence rhizosphere microbial community structure via selectively increasing the abundance of specific microbial groups, which in turn would influence plant P-acquisition. Finally, the effects of trait plasticity on plant-soil microbe interactions was indirectly investigated with the hypothesis that these interactions would be strongly mediated by soil type.

Material and methods

Experimental design

In order to examine the interactions between belowground functional traits involved in P-acquisition and fungal and bacterial communities, eight species (Brassicaceae: *Brassica carinata* A. Braun, *Raphanus sativus* L., *Sinapis alba* L.; Fabaceae: *Lens culinaris* Medik., *Pisum sativum* subsp. *Arvense* L., *Trifolium alexandrinum* L., *Vicia faba* L. and *Vicia sativa* L.) were grown under greenhouse conditions in two soil types. We selected species of two contrasting phylogenetic lineages in order to observe a gradient of belowground morphological and physiological P-acquisition traits and understand how their diverging P-acquisition strategies interact with microbial communities for P-acquisition. Brassicaceae were selected alongside Fabaceae for their different P-acquisition strategies and notably interactions with soil microorganisms, because Brassicaceae are predominantly non-mycorrhizal (Cosme et al. 2018). Soils with contrasting characteristics (Table 1), a Retisol (formerly called Albeluvisol) and a Calcaric Cambisol (characterized by the presence of an A horizon developed over a calcareous substrate) (FAO 2014), were collected from cultivated fields from north-eastern France. In order to further reduce P availability, soils were collected after topsoil (0-5 cm) removal and mixed with washed sand (22% of the final weight). After mixing, soil P concentrations were of 19.8 mg P kg⁻¹ and 16.7 mg kg⁻¹. Shoot P concentration of 0.8 to 3 mg P kg⁻¹ might indicate moderate P limitation. Pots were randomly arranged with two individuals per species per pot (4.5 L pots), and 2.5 months growing period. Greenhouse conditions were 22°C during the day (14 hours) and 18°C at night. The experiment was carried out in triplicate, with eight species and two soil types for a total of 48 pots.

Table 1 Chemical and physical soil characteristics

	% Clay (<0.002mm)	% Sand (>0.05mm)	pH KCl	Ca ^a (gkg ⁻¹)	N tot (mg kg ⁻¹)	CEC (cmol _c kg ⁻¹)	Olsen P ^c (mg kg ⁻¹)	Ca-P ^d (mg kg ⁻¹)
Calcaric	30.7	22.0	7.9	44.7 ^b	1700	9.3	19.8	542
Cambisol								
Retisol	22.3	5.4	7.4	2.60	1000	10.5	16.7	76.7
p value							NS	0.03

p value: Analysis of variance between soil for replicated conditions. NS : Not significant. Abbreviations: Ca: exchangeable calcium; N tot: total nitrogen; CEC: cation exchange capacity.

^aExtractant : Ammonium acetate 0.5 M, EDTA 0.02 M pH 4.65

^bCaCO₃ 56.4 %

^cAccording to Olsen (1954)

^dApatite phosphorus as defined in García-Albacete et al. (2012)

Plant measurements

Carboxylate release

At harvest, plants were carefully separated from the bulk soil, with care taken to avoid damage to the root system. Soil tightly adhering to the root system after shaking, thereafter described as rhizosphere as in Pang et al. (2017), was collected and stored at -20°C for further analysis. Carboxylate-release rate was measured by immersing the entire root system in 0.20 mM CaCl₂ for 1 hour under the same greenhouse conditions as used for plant growth, after rinsing off any remaining soil (Yacoumas et al. 2020). Reversed-phase column liquid chromatography was then used to measure carboxylate concentrations in the extract, offering a good resolution for five carboxylates (citrate, fumarate, maleate, malate, malonate). Briefly, a C18 column with a 15 min elution time and a 1 mL min⁻¹ flow rate was used with an acid mobile phase of 93% 25 mM KH₂PO₄ at pH 2.5 and 7% (v/v) methanol (Cawthray 2003; Yacoumas et al. 2020). The total carboxylate-release rate was calculated as the sum of all mentioned carboxylates.

Morphological traits

The plants were severed at the root-shoot junction for further measurements on the root system. All Fabaceae were normally nodulated. The root systems were scanned while being immersed in pure water using an Epson Scanner perfection V800 (Regent Instruments Inc., Québec, Qc, Canada) to produce a 600 dpi image. Root length, root surface area and root length per diameter class were determined using WinRHIZO Regular software V.2016a (Regent Instruments Inc., Québec, Qc, Canada). Root length density (RLD) was calculated based on 4.5 L pot volume, and the percentage of fine roots was calculated based on the percentage of root length of roots with a diameter less than 0.5 mm (Elshafie et al. 2013; Wang et al. 2015). After drying at 60°C for 48 hours, root dry weight was measured and specific root length (SRL) was calculated.

Phosphorus content

All aboveground biomass was dried at 60°C for 48 hours and weighed, before being digested via acid digestion and a microwave heating treatment (Lange et al. 2016). Briefly, 0.1 to 0.2 g of dry biomass was digested for one hour in a Teflon vessel with

8 mL of 65% (v/v) HNO₃ and 2 mL of 36% (v/v) HCl. After filtration, digest P and manganese (Mn) concentration, a proxy for rhizosheath carboxylate concentration (Pang et al. 2018), were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Scientific XSERIES2, Beauvais, France). A certified reference (SRM1573) was used as external standard.

Soil analyses

Acid phosphatase activity in the rhizosheath soil (PME) was determined by measuring the production of *p*-nitrophenol from sodium *p*-nitrophenyl phosphate, as described in Tabatabai and Bremner (1969). Briefly, three sub-samples of 0.5 g dry soil per replicate were added to a modified universal buffer at pH 6.5 with 0.2 mL toluene and 1 mL substrate (*p*-nitrophenyl phosphate). After one hour at 37°C, the reaction was stopped with 4 mL 0.5M NaOH and 1 mL 0.5 M CaCl₂. After filtering to remove the soil from the solution, *p*-nitrophenol release was determined spectrophotometrically at 410 nm. Soil pH was measured in the rhizosheath and bulk soil on 2 g equivalent dry soil with a 1:10 water to soil ratio. Δ pH between rhizosheath and bulk soil was then calculated.

Phosphorus availability

Microbial P, resin P and Olsen P in the rhizosheath were extracted before inorganic P determination on filtered extracts using malachite green colorimetry at 610 nm (Ohno and Zibilske 1991). Olsen P was extracted with a 1:20 soil to solution ratio and a solution of NaHCO₃ at pH=8.5 (Olsen 1954). Microbial P was determined via a differential method between hexanol-fumigated and non-fumigated extracts (Bunemann et al. 2007). After initial preparation with 0.5 M NaHCO₃, anion-exchange strips were shaken for 16 h with 2 g equivalent dry soil and 30 mL deionised water. For each sample, three portions of soil were extracted, with or without addition of 1 mL hexanol as fumigant and with spiking of a known amount of inorganic P (KH₂PO₄). The anion-exchange strips (VWR, 551642S) were then rinsed with deionised water before P was extracted by shaking 16 h in 20 mL 0.5 M HCl. To compensate for sorption of the P released after fumigation, P recovery was calculated in spiked samples otherwise extracted identically to non-fumigated samples. Microbial P was calculated as the difference between fumigated and non-fumigated samples, corrected by the recovery after spiking. To examine change in

rhizosheath P concentrations, Δ resin P, Δ microbial P and Δ Olsen P were calculated based on differences of concentration of resin P, microbial P and Olsen P between rhizosheath and bulk soil.

PLFA and NLFA determination

Frozen rhizosheath soil was freeze-dried for 48 hours. Any remaining root fragments were then manually removed before milling with an MM 300 Mixer Mill (Qiagen, City, USA). Phospholipid fatty acids (PLFA) were then extracted and analysed by the procedure of Frostegård et al. (1993). The total lipid extraction procedure followed that described by Frostegård et al. (1991), which is based on the method by Bligh & Dyer (1959) as modified by White et al. (1979). For detailed information on the PLFA and NLFA extraction see supplementary material S1. Specific PLFA and NLFA biomarkers, as highlighted in He et al. (2007), were summarised per main microbial group, that is bacteria, Gram-positive bacteria, Gram-negative bacteria, fungi and arbuscular mycorrhizal fungi. The NLFA/PLFA ratio of C16:1 ω 5 was calculated to account for possible bacterial interference when interpreting NLFA C16:1 ω 5 concentration as an indicator of AMF storage over growth, with possible interference being noted for a ratio less than 1. NLFA were used as indicators of recently dead bacterial necromass (Malosso 2004; Amir et al. 2008; Rinnan and Bååth 2009). The sum of Gram-negative NLFA and Gram-positive NLFA and the sum of Gram-negative PLFA and Gram-positive PLFA were calculated as indicators of total bacterial abundance (necromass and living biomass respectively).

Statistical analyses

All tests were performed with R v 3.6.0, the package plspm v 4.6, Rcmdr 2.5-3 and XLSTAT 2020.4.1.1018 at a significance level of 0.05. Differences between plant belowground P-acquisition traits, rhizosheath microbial indicators and P-acquisition indicators between families, soils, interactions between soils and families as well as species within families were first examined with nested ANOVAs in order to highlight their plasticity per soil type, family and intra-family variability. Differences between PLFA and NLFA and associated bacterial and fungal communities in the rhizosheath in the two soils were then examined via a principal component analysis (PCA) (supplementary material S2). As two distinct clusters regrouping all species per soil

type were identified on the PCA, further analysis was performed separately per soil type.

Interactions between traits, PLFA, NFLA and P-acquisition, as measured via total P content and change in rhizosheath P concentration (Resin P, Olsen P, Microbial P), were examined via either Pearson or Spearman correlation, according to the normality of the data. Generalized linear models (GLM) were then used to further identify the contribution of the two phylogenetic families examined, the two soil types as well as interactions between soil type and family to the main interactions highlighted. A regressor selection method was employed. Briefly simple models between one rhizosheath microbial indicator or indicator of P-acquisition and one plant belowground P-acquisition trait or rhizosheath microbial indicator were produced, specifying an effect of family and soil type as well as their interaction with said belowground P-acquisition trait or rhizosheath microbial indicator. Regressors were then progressively selected until only significant regressors were left in the model. Gamma family with inverse link functions was used.

Finally, to understand the combined influence of root traits and PLFA/NFLA on P-acquisition and synthesise the multiple correlations observed to further highlight the key variables involved, we used a partial least square path model (PLS PM) (Sanchez 2013). Three clusters of variables, or “latent variables” were defined: “belowground traits”, “PLFA/NFLA” and “P acquisition”. “Belowground traits” grouped change in rhizosheath pH, total carboxylate-release rate, citrate-release rate, fumarate-release rate, maleate-release rate, malate-release rate, malonate-release rate, leaf Mn concentration, phosphomonoesterase activity in the rhizosheath, root surface area, specific root length, root length density and fine root percentage. “PLFA/NFLA” grouped Fungi PLFA, Gram-positive PLFA, Gram-negative PLFA, bacterial PLFA, AMF NLFA, Fungal NLFA, Gram-positive NLFA, Gram-negative NLFA, bacterial NLFA, total PLFA and total NLFA. “P acquisition” grouped total P content, Δ Olsen P, Δ resin P and Δ microbial P. The “belowground traits” and “PLFA/NFLA” latent variables were then used to predict the “P acquisition” latent variable. To account for a possible influence of belowground traits on microbial communities, an interaction between the “belowground traits” latent variable and the NLFA/PLFA latent variable was specified in the model structure. To avoid dependency effects, replicates were averaged per species. Unidimensionality of

latent variables, as well as cross-loadings of variables within each latent variable were verified. To ensure positive correlations in a latent variable, the sign of some variables was modified. A first model incorporating all variables was used to select the most representative variables. Variables were selected based on their loadings (greater than 0.7) (Sanchez 2013). The PLFA/NFLA component was thus renamed as NFLA, after all PLFA indicators were eliminated when selecting variables. Models were produced with the “plsrm” package version 0.4.9 and evaluated based on their goodness of fit (GOF). Data deposited in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.rv15dv47s>, (Honvault et al. 2021).

Results

Differences among belowground traits, microbial communities and P-acquisition indicators per family, soil type and species

Strong differences were observed between Brassicaceae and Fabaceae notably for microbial indicators (Table 3) but also belowground traits (Table 4) and indicators of plant P uptake (Table 2) (supplementary material S3 and S4). AMF NLFA rhizosheath concentration and Gram-negative NLFA concentration were higher for Fabaceae, while nested effects of species per family were also observed. Brassicaceae had lower AMF NLFA rhizosheath concentration with ratios of concentration of C16:1 ω 5 neutral lipid and concentration of C16:1 ω 5 phospholipid inferior to one in both soils. The release rate malate and malonate were higher for the Fabaceae family with no significant differences between species per family for malate, while differences were observed for malonate. Fabaceae had higher P content overall, with shoot P concentration between 0.8 mg P kg⁻¹ and 3 mg P kg⁻¹ and higher decreases in rhizosheath Olsen P concentration. Differences per species per family were also noted both for plant P content and decreases in rhizosheath Olsen P concentration.

Multiple plant traits were different per soil type. Leaf Mn concentration was strongly different between soils, family and between species per family. Root malate and malonate-release rate were similarly different per soil. Root morphology also proved to be different per soil type, in particular fine root length percentage.

Table 2 Results of the nested ANOVA for the effect of soil, phylogenetic family, their interactions and nested effects of species within families on the changes in rhizosheath soil P concentration and plant P content

Source of variation	d.f.	Δ Resin P (mg/kg)		Δ Olsen P (mg/kg)		P content (mg)	
		Mean squares	F-values	Mean squares	F-values	Mean squares	F-values
Soil	1	517.23	22.94***	505.75	32.37***	42.30	3.10
Family	1	515.93	22.89***	304.89	19.52***	882.58	64.69***
Soil* Family	1	2.70	0.12	2.28	0.15	23.32	1.71
Specie(Family)	12	44.52	1.98	52.83	3.38**	75.40	5.53***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Abbreviations: Δ Resin P: Change in rhizosheath resin extracted P concentration (mg kg^{-1}); Δ Olsen P: Change in rhizosheath Olsen P concentration (mg kg^{-1}); P content : Total P content in plant biomass (mg P). For results for the other P-acquisition indicators measured see supplementary material S3.

Table 3 Results of the nested ANOVA for the effect of soil, phylogenetic family, their interactions and nested effects of species within families on rhizosheath PLFA and NLFA concentration

Source of variation	d.f.	PLFA				NLFA									
		Gram +		Bacterial		AMF		Fungi		Gram +		Gram -		Bacterial	
		MS	F-values	MS	F-values	MS	F-values	MS	F-values	MS	F-values	MS	F-values	MS	F-values
Soil	1	7.28	27.29***	25.83	6.75*	53.11	39.33***	0.03	0.25	0.85	14.94***	0.91	0.55	0.00	0.00
Family	1	0.16	0.62	16.37	4.28*	165.86	122.83***	0.78	6.46*	0.00	0.02	56.81	34.38***	57.32	31.12***
Soil* Family	1	0.18	0.69	6.72	1.76	30.26	22.41***	0.06	0.51	0.00	0.01	0.41	0.25	0.45	0.24
Specie(Family)	12	0.45	1.69	7.10	1.86	10.89	8.06***	0.26	2.16*	0.19	3.27**	3.85	2.33*	4.69	2.54*

* P< 0.05 ; ** P< 0.01; *** P<0.001. Abbreviations : PLFA : Phospholipid fatty acid; NLFA : Neutral lipid fatty acid; AMF: Concentration of C16:1 ω 5 neutral lipid; Fungi: Concentration of C18:2 ω 6,9 neutral lipid; Gram + : Sum of concentration of i15:0, a15:0, i16:0, i17:0, a17:0 phospholipids or neutral lipids; Gram - : Sum of concentration of cy17:0, C18:1 ω 7, cy:19:0 neutral lipids; Bacterial : Sum of Gram-positive and Gram-negative bacteria phospholipids or neutral lipids concentration; MS : mean squares. For results for the other PLFA and NLFA concentration measured see supplementary material S3.

Table 4 Results of the nested ANOVA for the effect of soil, phylogenetic family, their interactions and nested effects of species within families on belowground traits

Source of variation	d.f.	Malate		Malonate		FR		Leaf [Mn]		PME		RLD		RSA		TCR	
		MS	F-values	MS	F-values	MS	F-values	MS	F-values	MS	F-values	MS	F-values	MS	F-values	MS	F-values
Soil	1	221	6.26*	60	4.49*	0.04	27.72***	104429	71.10***	0.0042	29.87***	0.17	20.59***	75517	21.49***	1452	8.99**
Family	1	395	11.20**	418	31.36***	0.53	375.93***	63014	42.90***	0.0017	12.25**	0.01	1.64	42160	12.00**	1480	9.16**
Soil* Family	1	139	3.94	29	2.19	0.01	5.01*	26264	17.88***	0.0010	7.33**	0.01	0.79	1390	0.40	205	1.27
Specie(Family)	12	43	1.22	33	2.45*	0.10	69.74***	10770	7.33***	0.0002	1.52	0.04	4.41***	17905	5.10***	206	1.27

* P < 0.05 ; ** P < 0.01; *** P < 0.001. Abbreviations : Malate : Malate-release rate ($\mu\text{mol g root}^{-1} \text{hour}^{-1}$); Malonate : Malonate-release rate ($\mu\text{mol g root}^{-1} \text{hour}^{-1}$); FR: Fine root percentage (<0.5 mm diameter); Leaf [Mn] : Leaf manganese concentration ($\mu\text{g Mn g}^{-1}$); PME : Phosphomonoesterase activity in the rhizosheath ($\mu\text{g nitrophenol g}^{-1} \text{hour}^{-1}$); RLD : Root length density (m m^{-3}); RSA: Root surface area (cm^2); TCR: Total carboxylate-release rate ($\mu\text{mol g root}^{-1} \text{hour}^{-1}$); MS : mean squares. For results for the other belowground traits measured available in supplementary material S3.

Interaction between PLFA/NLFA microbial indicators, belowground traits and P-acquisition indicators

Interactions between carboxylate release rate and PLFA/NLFA microbial indicators

Significant correlations were observed between root carboxylate-release rates and NLFA fungal and bacterial indicators, with most correlation coefficients between 0.7 and 0.9 (Figures 1 and 2, supplementary Table S5). Carboxylate-release rate was strongly correlated with NLFA and PLFA concentrations in the rhizosheath, with malate-release rate in particular being positively correlated with multiple indicators such as AMF NLFA concentration in both soils (Figures 1a1,2a1) but also Gram-negative NLFA concentration in the Retisol (Figure 1b3). In the Calcaric Cambisol, malonate-release rate was positively correlated with Gram-negative NLFA concentration (Figure 2 b3), AMF NLFA concentration and bacterial NLFA concentration. Total carboxylate-release rate was not correlated with Gram-negative NLFA or AMF NLFA concentration in the Retisol, while it was positively correlated with AMF NLFA, fungi NLFA and Gram-negative NLFA concentrations in the Calcaric Cambisol. Generalized linear models reinforced these interactions with malate-release rate and malonate-release rate being significant predictors of Gram-negative NLFA concentration, AMF NLFA concentration and bacterial NLFA concentration (Table 5). Previously highlighted differences between families also contributed to these relationships, with interactions between malate-release rate and malonate-release rate and family being selected as predictors, while relationships between PLFA/NLFA microbial indicators and total carboxylate-release rate observed separately per soil were mostly associated with family effects overall. Gram-negative NLFA concentrations, bacterial NLFA and AMF NLFA concentrations were positively correlated with leaf Mn concentration in the Calcaric Cambisol (Figure 2 b4), and leaf Mn concentration was also positively correlated with total carboxylate-release. GLMs indicated significant relationships between leaf Mn concentration and these microbial indicators, but also interactions between leaf Mn concentration and family and soil type.

Interactions between root morphological traits and PLFA and NLFA indicators

Fewer correlations were observed between root morphological traits and bacterial and fungal communities. The percentage of fine roots was negatively correlated with

Gram-negative NLFA concentration in both soils (Figures 1b2, 2 b2), AMF NLFA and bacterial NLFA concentrations in the Retisol. Interactions between the percentage of fine roots and family as well as soil type also proved to be significant predictors of Gram-negative NLFA concentration, AMF NLFA and bacterial NLFA concentrations.

Interactions between changes in rhizosphere soil P concentration, plant P content and PLFA/NLFA indicators

We observed multiple correlations between plant P content or changes in the rhizosphere P concentration and PLFA and NLFA concentrations. Phosphorus content was strongly positively correlated with Gram-negative NLFA concentration (Figures 1b1, 2 b2) and bacterial NLFA concentration in both soils, and also with AMF NLFA concentration in the Retisol. Δ resin P and Δ Olsen P were similarly correlated with Gram-negative PLFA, AMF NLFA, Gram-negative NLFA and bacterial NLFA concentrations. The negative correlation between these variables indicates higher decreases in P availability in the rhizosphere associated with higher value of these indicators. Interactions between microbial indicators and family and soil type proved to be significant predictors of plant P content, while soil type and family alone also proved to be significant predictors.

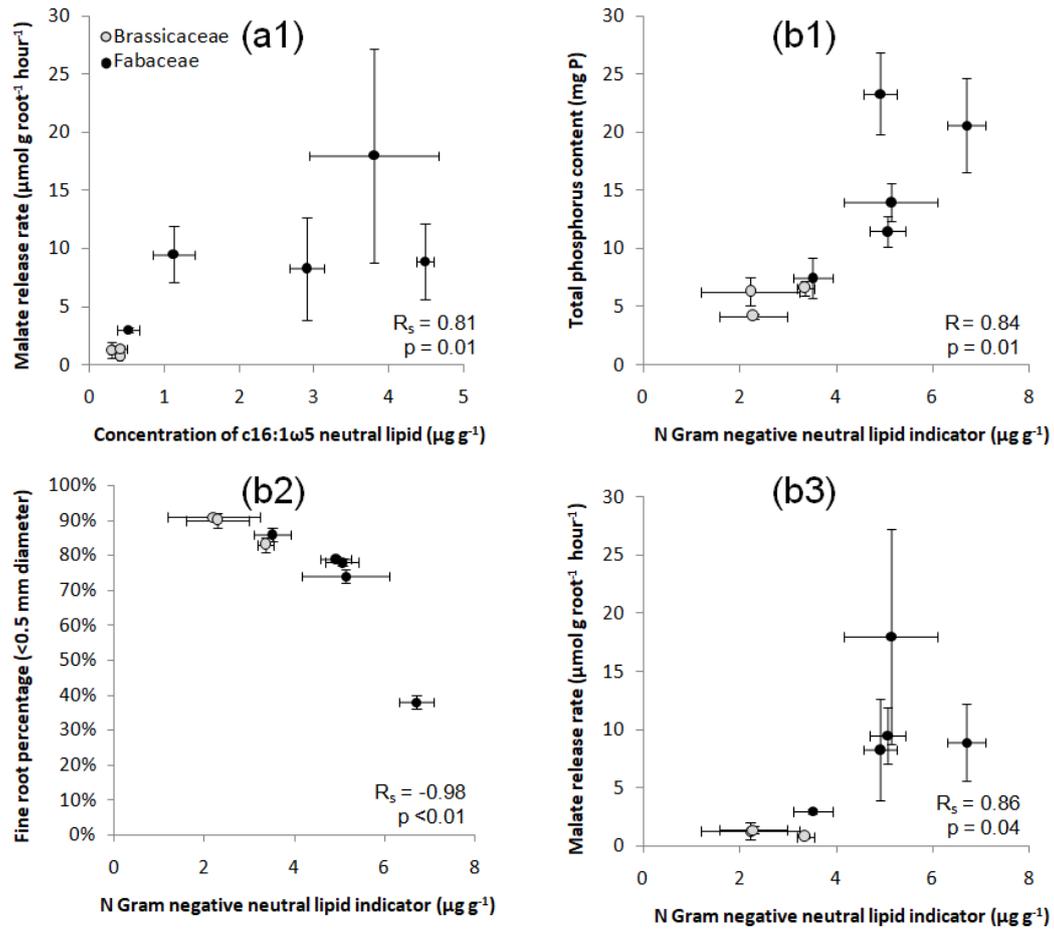


Figure 1 Correlations between rhizosheath fungal and bacterial NLFA concentrations, belowground traits and plant phosphorus P-content in the Retisol.

a Correlations between AMF NLFA indicator and **1** Malate-release rate; **b** Correlations between Gram-negative NLFA indicator and **1** Total plant P content; **2** Fine root percentage; **3** Malate-release rate.. R: Pearson correlation coefficient. R_s : Spearman correlation coefficient. p : p-value of the corresponding correlation test. Brassicaceae are represented in light gray, Fabaceae in black.

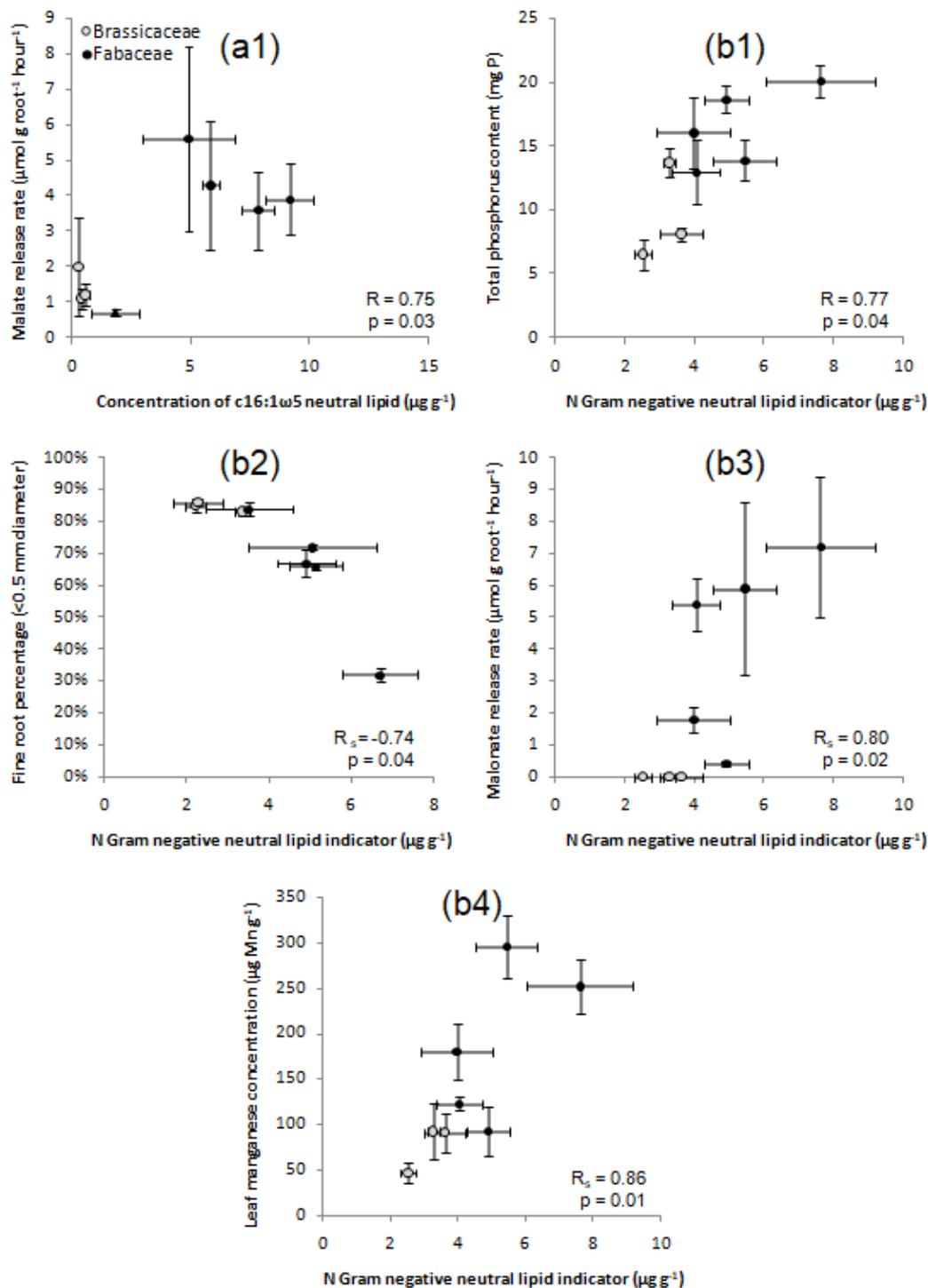


Figure 2 Correlations between rhizosphere fungal and bacterial NLFA concentrations, belowground traits and plant P-content in the Calcaric Cambisol.

a Correlations between AMF NLFA and 1 Malate-release rate; rhizosphere. **b** Correlations between the Gram-negative NLFA indicator and 1 Total plant P-content; 2 Fine root percentage; 3 Malonate-release rate; 4 Leaf manganese (Mn) concentration. R: Pearson correlation coefficient. R_s : Spearman correlation coefficient. p : p value of the corresponding correlation test. Brassicaceae are represented in light gray, Fabaceae in black.

Table 5 Selected models fitted to rhizosheath fungal and bacterial NLFA concentrations and plant P content

Variable predicted	Predictor ¹	Selected regressors ¹	Pr(> t)
Gram-negative NLFA	Malate	Malate	0.002**
		Soil	0.004**
		Malate * Family	0.002**
	Malonate	Malonate	0.001**
		Family	0.000***
		Malonate * Family	0.001**
	TCR	Family	0.000***
	Leaf [Mn]	Leaf [Mn]	0.000***
		Family	0.000***
		Soil	0.001***
		Leaf [Mn] * Family	0.000***
		Leaf [Mn] * Soil	0.008**
		FR	FR
	FR	Family	0.007**
		Soil	0.022*
FR * Family		0.003**	
FR * Soil		0.025*	
Malate		Malate	0.001***
Bacterial NLFA	Malate	Malate * Family	0.001***
		Malonate	0.000***
	Malonate	Family	0.000***
		Malonate * Family	0.000***
	TCR	Family	0.000***
	Leaf [Mn]	Leaf [Mn]	0.000***
		Family	0.000***
		Soil	0.000***
		Leaf [Mn] * Family	0.000***
		Leaf [Mn] * Soil	0.004**
FR		Fine root length %	0.002**
FR	Family	0.006**	
	Soil	0.012*	
	FR * Family	0.003**	
	FR * Soil	0.034*	
	Malate	Soil	0.006**
AMF NLFA	Malonate	Family	0.000***
		Malonate * Sol	0.000***
	TCR	TCR	0.005**
		Family	0.000***
		TCR * Soil	0.002**
	Leaf [Mn]	Family	0.000***
		Leaf [Mn] * Soil	0.035*
	FR	Family	0.000***
		FR * Soil	0.001***

Plant P content	Gram-negative NLFA	Family	0.003**
		Soil	0.004**
		Gram-negative NLFA * Soil	0.007**
	Bacterial NLFA	Family	0.002**
		Soil	0.006**
		Bacterial NLFA * Soil	0.012*

* P < 0.05 ; ** P < 0.01; *** P < 0.001. ¹ : For model fitting initial model structure was Predicted ~ Predictor + Family + Soil + Predictor * Family + Predictor * Soil. ²: Selected based on a significance of the variable, factor or interaction term with a significance level of 0.05 Abbreviations : Malate : Malate-release rate ($\mu\text{mol g root}^{-1} \text{hour}^{-1}$); Malonate : Malonate-release rate ($\mu\text{mol g root}^{-1} \text{hour}^{-1}$); FR: Fine root percentage (<0.5 mm diameter); Leaf [Mn] : Leaf manganese concentration ($\mu\text{g Mn g}^{-1}$); TCR: Total carboxylate-release rate ($\mu\text{mol g root}^{-1} \text{hour}^{-1}$); AMF NLFA: Concentration of C16:1 ω 5 neutral lipid; Gram-negative NLFA : Sum of concentration of cy17:0, C18:1 ω 7, cy:19:0 neutral lipids; Bacterial NLFA: Sum of Gram-positive and Gram-negative bacteria neutral lipids concentration; Plant P content : Total P content in plant biomass (mg P).

Modelling P-acquisition via interaction between PLFA and NLFA and root traits

Interactions between belowground traits and NLFA indicators in predicting P-acquisition

PLS-PM offered a good fit when predicting P-acquisition as measured via total P content and ΔP concentrations based on belowground traits and NLFA indicators, with a GOF between 0.60 and 0.82 (Figure 3). We observed strong positive correlations between the “belowground traits” latent variable and the “NLFA” latent variable (Corr= 0.68 and 0.88 in the Calcaric Cambisol and Retisol, respectively). Correlation between the “belowground traits” latent variable and the “NLFA” latent variable resulted in a slightly lower indirect positive effect of belowground traits on P-acquisition via their effect on NLFA (Corr= 0.34) than the direct positive effect of the “NLFA” latent variable on P-acquisition (Corr= 0.38) in the Retisol (supplementary material S6). In the Calcaric Cambisol, the latent variables “belowground traits” and “NLFA” presented equal correlations (Corr= 0.37) with the latent variable “P acquisition”. In the Retisol the latent variable “belowground traits” presented a stronger correlation with the latent variable “P acquisition (Corr= 0.54), while the latent variable “NLFA” had a similar correlation coefficient to the one observed in the Calcaric Cambisol (Corr= 0.38).

Contribution of microbial indicators to the “NLFA” latent variable

The “NLFA” latent variable was accurately described (ρ = 0.97 and 0.98) by similar key variables in both soils, namely AMF NLFA, Gram-negative NLFA and bacterial NLFA (supplementary material S7). The NLFA latent variable was mainly represented by the Gram-negative NLFA variable, followed by the bacterial NLFA variable indicating that most of the variation in NLFA concentrations was observed for these variables.

Contribution of root traits to the “Root traits” latent variable

The traits best describing the “Root traits” latent variable (ρ = 0.84 and 0.94) differed between soils. Two morphological traits, fine root percentage and root surface area, were selected in the Calcaric Cambisol while none were in the Retisol. Malate-release rate strongly contributed to the “belowground traits” latent variable in both soils, while total carboxylate-release rate described the latent variable well in

the Calcaric Cambisol, but not in the Retisol. Another physiological indicator, phosphomonoesterase activity best described it in the Retisol.

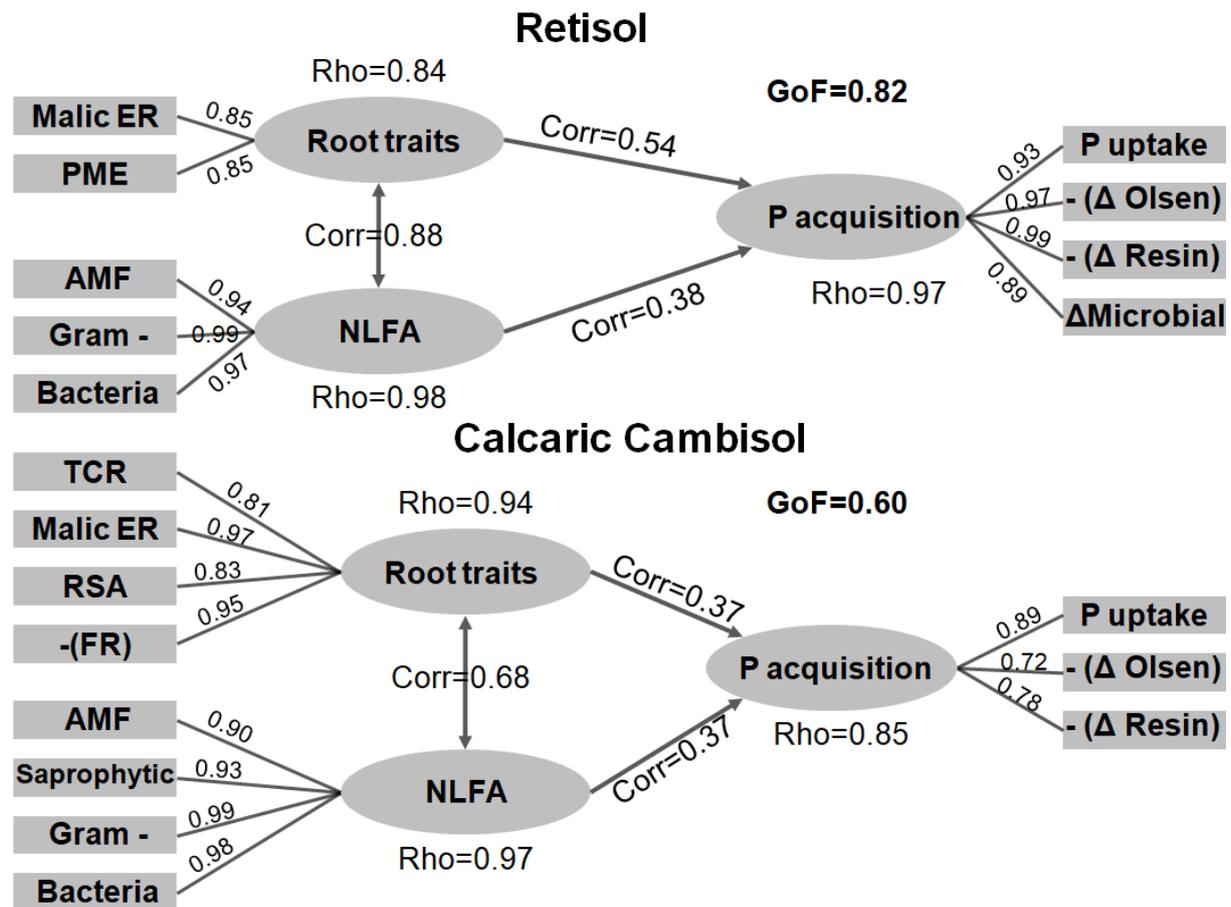


Figure 3 : Partial least square-path models predicting phosphorus (P) acquisition

Corr is the correlation effect, Rho the Dilon-Golstein coefficient. Variables negatively transformed indicated by -(variable). GoF= Goodness of Fit of the model. Based on three latent variables “Belowground traits”, “NLFA” and “P acquisition” (in ovals), which are constructs based on multiple individual factors (in square boxes). Individual factors loading presented per latent variable. Abbreviations: Δ Microbial: difference in microbial P concentration between rhizosheath and bulk soil ; Δ Olsen: difference in Olsen P concentration between rhizosheath and bulk soil; Δ Resin: difference in resin P concentration between rhizosheath and bulk soil; FR : % fine roots (<0.5 mm); Malate : malate-release rate ; PME : phosphomonoesterase activity; RSA : root surface area; TCR : total carboxylate-exudation rate; AMF: Concentration of C16:1ω5 neutral lipid; Gram - : Sum of concentrations of cy17:0, C18:1ω7, cy:19:0 neutral lipid; Fungi : Concentration of C18:2ω6,9 neutral lipid; Bacteria: Sum of N Gram-negative and N Gram-positive; NLFA: Neutral lipid fatty acid.

Discussion

Interaction between root morphology and rhizosphere microbial communities for P-acquisition

Plant-soil microorganism interactions play a major role in nutrient cycling and ecosystem functioning (Richardson and Simpson 2011; Richardson et al. 2011; Sunita 2017; Nobile et al. 2019). Despite recognition of the importance of such plant-soil microorganism interactions the role of specific plant functional traits in the mechanisms driving rhizosphere fungal and bacterial communities and their effect on plant P-acquisition has rarely been investigated (Bardgett et al. 2014; Bardgett 2017; Datta et al. 2020). Our study provide evidence for strong relationships between several P-acquisition traits and fungal and bacterial rhizosphere communities composition, and in turn plant P uptake. Fine root percentage was negatively correlated with Gram-negative NLFA indicators in both soils, and with AMF NLFA indicators in the Retisol. The several Brassicaceae species examined in this study, which are non-mycorrhizal, may in part explain these correlations as evidenced by the GLMs produced highlighting family as a significant predictor of AMF NLFA indicators. A relationship between fine root percentage and AMF NLFA indicators as modulated by soil type was, however, also highlighted reinforcing a likely link between fine root percentage and AMF NLFA indicators apart from phylogenetic patterns. Fine root percentage similarly proved to be a significant predictor of Gram-negative NLFA indicators, while family, soil type and interactions with these factors also contributed to the overall relationship. This suggest that while phylogeny in part drove relationships between Gram-negative NLFA indicators and fine root percentage a direct relationship between these variable is likely. Plants with thinner roots in turn presented smaller decrease in rhizosphere resin P or Olsen P compared to the bulk soil (in the Calcaric Cambisol and the Retisol, respectively). While potentially exploiting a large soil volume at a reduced cost, they captured less of the available P in their rhizosphere, possibly due to reduced bacterial associations. Plants with thin roots have been observed to rely less on associations with arbuscular mycorrhizal fungi for P-acquisition, and rather rely on enhanced soil P foraging through higher SRL or root branching (Zhou et al. 2018; Wen et al. 2019). A “collaboration gradient” has been proposed to explain the relationships often

observed between root diameter and association with Fungi with the hypothesis that plants adjust their nutrient foraging strategies along this axis (Bergmann et al. 2020), which our findings showing a negative tendency between fine root percentage and AMF NLFA indicators appear to reinforce. Moreover, trade-offs between root diameter and other root functional traits involved in P-acquisition such as carboxylate-release or phosphomonoesterase activity in the rhizosphere, have also been reported in numerous species as a consequence of the carbon cost associated with these diverging P-acquisition strategies (Lyu et al. 2016; Wen et al. 2019). The pattern highlighted here of lower concentration of bacterial indicators in the rhizosphere of thinner roots suggests that these tradeoffs could also extend to associations with rhizosphere bacteria. Bacterial NLFA have, however, been suggested to be indicators of recently dead rather than living bacterial biomass (Rinnan and Bååth 2009; Malosso 2004; Amir et al. 2008), suggesting important bacterial turnover. In summary, we report plausible tradeoffs between root diameter and associations with microbes in the rhizosphere, and observed a smaller decrease of available P in the rhizosphere of thinner roots, which are less associated with soil microbial communities. Further studies incorporating other root morphological traits such as root-hair length or abundance and measuring pools of less plant-available soil P could provide valuable insights into the complex and still unclear interactions between root morphology and rhizosphere microbial communities for P-acquisition from multiple soil P pools.

Carboxylate exudation role in plant-microorganism interactions for P-acquisition

Differential exudation may play a major role in modulating plant interactions with soil microorganisms, as recently reviewed in Sasse et al. (2018). Carboxylates, a major fraction of total root exudates, can act as carbon source for microorganisms (Sasse et al. 2018), while also promoting P-acquisition via P mobilisation (Wang and Lambers 2020). Understanding the still enigmatic role of carboxylate-release in shaping microbial communities and its implications for P-acquisition is as such central to unravelling plant P-acquisition strategies. Our results demonstrate relationships between the release of carboxylates and rhizosphere community structure and could expand our understanding of the plausible role of specific carboxylates in plant-microorganism interactions for P-acquisition. Specifically, we

observed significant links between the release rate of malate, malonate and rhizosphere fungal and bacterial community composition. Both Gram-negative and AMF NLFA indicators increased in concentration with increasing malate-release rate and malonate-release rate. These relationships may have been driven by the phylogenetic families examined, as evidenced by the lack of significant differences among species per family for malate-release rate for example or by the interactions terms with soil type and family predicting Gram-negative and AMF NLFA indicators. GLMs however also confirmed that both malate-release rate and malonate-release rate were significant predictors of Gram-negative NLFA and AMF NLFA indicators. Our results thus hint at a potential role of released carboxylates and in particular malate and malonate in interacting with rhizosphere microbial communities. This potential role of carboxylates in shaping rhizosphere microbial communities is reinforced by the proposition made by Habekost et al. (2008) that the greater abundance of Gram-negative bacteria observed in the rhizosphere of Fabaceae may in turn be the result of more root exudation including but not limited to exudation of carboxylates. Numerous exudates have been identified to play a selective or inhibitor role in shaping rhizosphere microbial communities (More et al. 2019), while the specific contribution of carboxylates as compared with other exudates such as flavonoids remains to be fully investigated (Martin et al. 2016). The abundance of Gram-negative bacteria tends to be favoured by low-molecular-weight carbon sources (Prashar et al. 2014), and carboxylates have thus been proposed to be key drivers of rhizosphere soil microbial community composition (Hunter et al. 2014). The rhizosphere soil microbial community is, however, to be the result of more than just the utilization of carbon sources with multiple overlapping mechanisms involved (Hartmann et al. 2009). Our observation of strong correlations between carboxylate-release and Gram-negative bacteria indicators could indicate that irrespectively from phylogenetic patterns and exudates other than carboxylates, plants may have influenced their rhizosphere microbial communities by carboxylates exudation, reinforcing the role of carboxylate in plant-microbe interactions. Several carboxylates have indeed been observed to have considerable effects on rhizosphere microbial communities. Malate and malonate for instance has been observed to affect microbial community structure and activity (Marschner et al. 2002; Martin et al. 2016). In the present study correlations between malate or malonate and bacterial and fungal NLFA appear to strengthen and expand these observations to other

carboxylates. Shifts in microbial communities were in turn strongly related to plant P uptake, as indicated by positive correlations between plant P content and Gram-negative NLFA indicators. A significant fraction of rhizosheath bacteria comprises P-solubilising bacteria or plant-growth-promoting bacteria (Kalayu 2019). As such, shifts in microbial community structure possibly due to carboxylate-release by roots may increase P-acquisition. For instance, carboxylates promote root colonization by plant-growth-promoting bacteria (Yuan et al. 2015). Positive correlations between indicators of Gram-negative bacteria and plant P content, as well as with decrease in rhizosheath P concentration in our results could thus suggest the modulation of rhizosheath bacterial communities by plants for P-acquisition via carboxylate-release, while the extent of which plant control microbial communities and its plasticity according to soil type remains unclear. The results indeed suggested that while significant relationships exist between plant P content and rhizosheath bacterial indicators, these relationships are strongly modulated by soil type and are in part driven by phylogenetic patterns. Fabaceae indeed tended to have greater P content and result in higher decrease in rhizosheath concentration while also releasing more carboxylates, which contributed to these correlations in our experiment. By highlighting interactions between carboxylate-release by plants and rhizosheath fungal and microbial communities, driven in part by phylogeny while being also coherent with the current understanding of plant-soil microorganisms interactions, our new findings support the sparsely-reported role of carboxylate-release in shaping microbial communities and its implications for P-acquisition. As our results show that these interactions depend on soil type and properties future studies examining how soil biotic and abiotic factors drive the influence of specific carboxylates on soil microorganisms are needed. Moreover the relative contribution of carboxylates to these interactions as part of the wide range of root exudates also require more attention.

Soil type mediate plant-soil microorganism interactions involved in plant P-acquisition

The efficiency of several P-acquisition traits such as carboxylate-release or rhizosheath acidification as well as plant associations with mycorrhizal fungi is highly dependent on soil properties, especially P availability (Wen et al. 2019; Wang and Lambers 2020). Root traits values and their efficiency in acquiring P can indeed be

very variable due to plasticity induced by biotic and abiotic stress, with poorly understood consequences for rhizosheath microbial communities (Sweeney et al. 2021). Furthermore plant nutrient requirements are believed to be key factors driving the effects of plants on rhizosheath microbial communities (Datta et al. 2020), highlighting the need to expand our understanding of the role of trait plasticity in plant-microbe interactions for nutrient acquisition. In order to investigate the influence of soil types and properties on trait responses and interactions with microorganisms for P-acquisition we examined two contrasting soil types in our study. The results highlighted important modulation of plant-soil microorganism interactions by soil type, notably via relationships between different belowground traits or microbial communities and P uptake per soil type. In particular, leaf Mn concentration, an indicator of P mobilisation involving carboxylate-release (Pang et al. 2018), was correlated with AMF NLFA, Gram-negative NLFA and bacterial NLFA in the Calcaric Cambisol, but not in the Retisol. GLMs relating these factors similarly highlighted interactions between leaf Mn and soil type as significant predictors of AMF NLFA, Gram-negative NLFA and bacterial NLFA. Leaf Mn concentration was also correlated with total carboxylate-release rate in the Calcaric Cambisol, but not in the Retisol. Interestingly, we observed no correlation between leaf Mn concentration and total carboxylate-release rate corrected for the number of carboxylic groups per carboxylate. Our results may thus indicate a different role of released carboxylates in plant-soil microorganism interactions for P-acquisition dependent on soil properties, although an influence of soil Mn, not measured in our experiment, can not be ruled out as highlighted by the soil effects observed. In the Calcaric Cambisol, correlations between leaf Mn concentration and bacterial NLFA indicators suggest that the released carboxylates were effective at mobilising P which is reinforced by the correlation between total carboxylate-release and leaf Mn concentration, but also partly metabolized by bacteria. Bacteria could also have contributed to this observation, as Mn availability in soil is strongly affected by microbes which in turn depend on root exudates as carbon source (More et al. 2019). In the Retisol, the lack of correlation between microbial indicators and leaf Mn concentration could indicate rapid microbial consumption upon release, as shown before (D'Angioli et al. 2017). Increased microbial abundance due to carboxylate consumption would, in turn, increase plant P-acquisition through positive plant-microorganisms feedback, explaining the slightly higher correlation coefficient between microbial indicators and

indicators of plant P-acquisition in this soil, although higher P content in Fabaceae may affect this observation. The complex combination of carboxylate-release for P mobilisation and microbial consumption of exudates has indeed been suggested to depend on the prevailing soil conditions and microbial community (Hunter et al. 2014). In our study, the differences in carboxylate exudations and relationships between plant traits and microbial indicators per soil type for plants with overall similar P content between soils seem to reinforce this suggestion. The degree to which this plasticity in carboxylate exudations is controlled by plants in order to interact with microbial communities, rather than a consequence of root plasticity induced by soil properties, however, remains unclear (Sweeney et al. 2021). The increased P availability in soil with high Ca-P concentration can be explained by the weathering effect of carboxylate on minerals such as apatite as well as the mobilisation of Ca from the Ca-P surface and the Ca chelation by carboxylates, which, in turn, promotes the dissolution of Ca-P (Jones and Darrah 1994; Ryan et al. 2001; Rosling et al. 2007). We report how soil properties may affect plant-microorganism interactions for P-acquisition, with the carboxylates released most likely playing a different role in interacting with soil microbial communities and mobilizing P dependent on soil type.

Conclusion

Examining interactions between belowground functional traits and fungal and bacterial communities by measuring PLFA and NLFA concentrations in the rhizosheath allowed us to gain a new understanding of the role of plant-soil microorganism interactions in P-acquisition and the factors involved. We demonstrate that plant functional traits are strongly linked with rhizosheath fungal and bacterial communities, which are in return strongly involved in P-acquisition, although some of the interactions highlighted may be constitutive of Brassicaceae and Fabaceae species. Our results mainly highlighted specific links between bacterial communities and the release of carboxylates, namely malate and malonate, with the microbial groups affected seemingly playing an important role in P-acquisition. Differences in plant trait interactions with PLFA and NLFA between soil types suggested that soil type may mediate the role of carboxylates for P-acquisition. Perspectives include examining the carbon costs of plant-soil microorganism

interactions for P-acquisition via differential root exudation and clarify the relative contribution of carboxylate-release to these interactions and the plausible tradeoffs with other root traits involved in P-acquisition. The mechanisms involved in the relationships between soil properties and plant-soil microorganism interactions should moreover be investigated over a wide soil gradient where pH and P forms vary to elucidate the role of trait plasticity and in particular intraspecific variation of traits in these interactions and overall in P-acquisition by plants and ecosystem functioning.

References

- Adeleke R, Nwangburuka C, Oboirien B (2017) Origins, roles and fate of organic acids in soils: A review. *South African Journal of Botany* 108:393–406.
<https://doi.org/10.1016/j.sajb.2016.09.002>
- Amir S, Merlina G, Pinelli E, et al (2008) Microbial community dynamics during composting of sewage sludge and straw studied through phospholipid and neutral lipid analysis. *Journal of Hazardous Materials* 159:593–601.
<https://doi.org/10.1016/j.jhazmat.2008.02.062>
- Baath E (2003) The Use of Neutral Lipid Fatty Acids to Indicate the Physiological Conditions of Soil Fungi. *Microbial Ecology* 45:373–383.
<https://doi.org/10.1007/s00248-003-2002-y>
- Barberán A, McGuire KL, Wolf JA, et al (2015) Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. *Ecol Lett* 18:1397–1405.
<https://doi.org/10.1111/ele.12536>
- Bardgett RD (2017) Plant trait-based approaches for interrogating belowground function. *Biology and Environment: Proceedings of the Royal Irish Academy* 117B:1. <https://doi.org/10.3318/bioe.2017.03>
- Bardgett RD, Mommer L, De Vries FT (2014) Going underground: root traits as drivers of ecosystem processes. *Trends in Ecology & Evolution* 29:692–699.
<https://doi.org/10.1016/j.tree.2014.10.006>
- Bergmann J, Weigelt A, van Der Plas F, et al (2020) The fungal collaboration gradient dominates the root economics space in plants. *Science Advances* 6:eaba3756
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37:7

- Bunemann E, Marschner P, Mcneill A, Mclaughlin M (2007) Measuring rates of gross and net mineralisation of organic phosphorus in soils. *Soil Biology and Biochemistry* 39:900–913. <https://doi.org/10.1016/j.soilbio.2006.10.009>
- Campos P, Borie F, Cornejo P, et al (2018) Phosphorus Acquisition Efficiency Related to Root Traits: Is Mycorrhizal Symbiosis a Key Factor to Wheat and Barley Cropping? *Front Plant Sci* 9:752. <https://doi.org/10.3389/fpls.2018.00752>
- Cawthray GR (2003) An improved reversed-phase liquid chromatographic method for the analysis of low-molecular mass organic acids in plant root exudates. *Journal of Chromatography A* 1011:233–240. [https://doi.org/10.1016/S0021-9673\(03\)01129-4](https://doi.org/10.1016/S0021-9673(03)01129-4)
- Cosme M, Fernández I, Van der Heijden MG, Pieterse CM (2018) Non-mycorrhizal plants: the exceptions that prove the rule. *Trends in plant science* 23:577–587
- D'Angioli AM, Viani RAG, Lambers H, et al (2017) Inoculation with *Azospirillum brasilense* (Ab-V4, Ab-V5) increases *Zea mays* root carboxylate-exudation rates, dependent on soil phosphorus supply. *Plant Soil* 410:499–507. <https://doi.org/10.1007/s11104-016-3044-5>
- Datta R, Meena RS, Pathan SI, Ceccherini MT (eds) (2020) *Carbon and Nitrogen Cycling in Soil*. Springer Singapore, Singapore
- Defrenne CE, Philpott TJ, Guichon SHA, et al (2019) Shifts in Ectomycorrhizal Fungal Communities and Exploration Types Relate to the Environment and Fine-Root Traits Across Interior Douglas-Fir Forests of Western Canada. *Front Plant Sci* 10:643. <https://doi.org/10.3389/fpls.2019.00643>
- Eisenhauer N, Lanoue A, Strecker T, et al (2017) Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Sci Rep* 7:44641. <https://doi.org/10.1038/srep44641>
- Elshafie H, I C, E V, et al (2013) Use of plant growth promoting bacteria (PGDB) for promoting tomato growth and its evaluation as biological control agent. *Int J of Micr Res* 5:452–457. <https://doi.org/10.9735/0975-5276.5.5.452-457>

- FAO (2014) World reference base for soil resources 2014: international soil classification system for naming soils and creating legends for soil maps. FAO, Rome
- Fort F (2013) Stratégies d'acquisition des ressources des plantes prairiales sous contraintes hydrique et minérale-Rôle du système racinaire dans la réponse aux facteurs structurant les communautés
- Frostegård Å, Tunlid A, Bååth E (1993) Phospholipid Fatty Acid Composition, Biomass, and Activity of Microbial Communities from Two Soil Types Experimentally Exposed to Different Heavy Metals. *Applied and Environmental Microbiology* 59:3605–3617.
<https://doi.org/10.1128/AEM.59.11.3605-3617.1993>
- Frostegård Å, Tunlid A, Bååth E (1991) Microbial biomass measured as total lipid phosphate in soils of different organic content. *Journal of Microbiological Methods* 14:151–163. [https://doi.org/10.1016/0167-7012\(91\)90018-L](https://doi.org/10.1016/0167-7012(91)90018-L)
- García-Albacete M, Martín A, Cartagena MC (2012) Fractionation of phosphorus biowastes: Characterisation and environmental risk. *Waste Management* 32:1061–1068. <https://doi.org/10.1016/j.wasman.2012.02.003>
- Gómez-Suárez AD, Nobile C, Faucon M-P, et al (2020) Fertilizer Potential of Struvite as Affected by Nitrogen Form in the Rhizosphere. *Sustainability* 12:2212. <https://doi.org/10.3390/su12062212>
- Habekost M, Eisenhauer N, Scheu S, et al (2008) Seasonal changes in the soil microbial community in a grassland plant diversity gradient four years after establishment. *Soil Biology and Biochemistry* 40:2588–2595.
<https://doi.org/10.1016/j.soilbio.2008.06.019>
- Haling RE, Brown LK, Stefanski A, et al (2018) Differences in nutrient foraging among *Trifolium subterraneum* cultivars deliver improved P-acquisition efficiency. *Plant Soil* 424:539–554. <https://doi.org/10.1007/s11104-017-3511-7>
- 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>

Harrison KA, Bardgett RD (2010) Influence of plant species and soil conditions on plant-soil feedback in mixed grassland communities. *Journal of Ecology* 98:384–395. <https://doi.org/10.1111/j.1365-2745.2009.01614.x>

Hartmann A, Schmid M, van Tuinen D, Berg G (2009) Plant-driven selection of microbes. *Plant Soil* 23

He Y, Xu J, Ma Z, et al (2007) Profiling of PLFA: Implications for nonlinear spatial gradient of PCP degradation in the vicinity of *Lolium perenne* L. roots. *Soil Biology and Biochemistry* 39:1121–1129. <https://doi.org/10.1016/j.soilbio.2006.11.023>

Hinsinger P, Herrmann L, Lesueur D, et al (2018) Impact of Roots, Microorganisms and Microfauna on the Fate of Soil Phosphorus in the Rhizosphere. In: Roberts JA (ed) *Annual Plant Reviews online*. John Wiley & Sons, Ltd, Chichester, UK, pp 377–407

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

Honvault N, et al. (2021). Data from: Interactions between belowground traits and rhizosphere fungal and bacterial communities for phosphorus acquisition. Dryad Digital Repository. <https://doi.org/10.5061/dryad.rv15dv47s>

Hou E, Chen C, Luo Y, et al (2018) Effects of climate on soil phosphorus cycle and availability in natural terrestrial ecosystems. *Glob Change Biol* 24:3344–3356. <https://doi.org/10.1111/gcb.14093>

Hu L, Robert CAM, Cadot S, et al (2018) Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nat Commun* 9:2738. <https://doi.org/10.1038/s41467-018-05122-7>

- 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:
<https://doi.org/10.3389/fpls.2014.00027>
- Jones DL, Darrah PR (1994) Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant Soil* 166:247–257.
<https://doi.org/10.1007/BF00008338>
- Kalayu G (2019) Phosphate Solubilizing Microorganisms: Promising Approach as Biofertilizers. *International Journal of Agronomy* 2019:1–7.
<https://doi.org/10.1155/2019/4917256>
- Lange B, Pourret O, Meerts P, et al (2016) Copper and cobalt mobility in soil and accumulation in a metallophyte as influenced by experimental manipulation of soil chemical factors. *Chemosphere* 146:75–84.
<https://doi.org/10.1016/j.chemosphere.2015.11.105>
- Legay N, Baxendale C, Grigulis K, et al (2014) Contribution of above- and below-ground plant traits to the structure and function of grassland soil microbial communities. *Annals of Botany* 114:1011–1021.
<https://doi.org/10.1093/aob/mcu169>
- Li H, Liu B, McCormack ML, et al (2017) Diverse belowground resource strategies underlie plant species coexistence and spatial distribution in three grasslands along a precipitation gradient. *New Phytol* 216:1140–1150.
<https://doi.org/10.1111/nph.14710>
- 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* 7:15.
<https://doi.org/10.3389/fpls.2016.01939>
- Ma Z, Guo D, Xu X, et al (2018) Evolutionary history resolves global organization of root functional traits. *Nature* 555:94–97. <https://doi.org/10.1038/nature25783>

- Malosso E (2004) Use of ¹³C-labelled plant materials and ergosterol, PLFA and NLFA analyses to investigate organic matter decomposition in Antarctic soil. *Soil Biology and Biochemistry* 36:165–175.
<https://doi.org/10.1016/j.soilbio.2003.09.004>
- Marschner P, Neumann G, Kania A, Weiskopf L (2002) Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupin (*Lupinus albus* L.). *246:167–174*
- Martin BC, George SJ, Price CA, et al (2016) Citrate and malonate increase microbial activity and alter microbial community composition in uncontaminated and diesel-contaminated soil microcosms. *SOIL* 2:487–498.
<https://doi.org/10.5194/soil-2-487-2016>
- 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>
- Micallef SA, Shiaris MP, Colón-Carmona A (2009) Influence of *Arabidopsis thaliana* accessions on rhizobacterial communities and natural variation in root exudates. *Journal of Experimental Botany* 60:1729–1742.
<https://doi.org/10.1093/jxb/erp053>
- More SS, Shinde SE, Kasture MC (2019) Root exudates a key factor for soil and plant: An overview
- 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>
- Nottingham AT, Turner BL, Stott AW, Tanner EVJ (2015) Nitrogen and phosphorus constrain labile and stable carbon turnover in lowland tropical forest soils. *Soil Biology and Biochemistry* 80:26–33.
<https://doi.org/10.1016/j.soilbio.2014.09.012>

- Ohno T, Zibilske LM (1991) Determination of Low Concentrations of Phosphorus in Soil Extracts Using Malachite Green. *Soil Science Society of America Journal* 55:892. <https://doi.org/10.2136/sssaj1991.03615995005500030046x>
- Olsen SR (1954) Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. U.S. Department of Agriculture
- Pang J, Bansal R, Zhao H, et al (2018) The carboxylate-releasing phosphorus-mobilizing strategy can be proxied by foliar manganese concentration in a large set of chickpea germplasm under low phosphorus supply. *New Phytol* 219:518–529. <https://doi.org/10.1111/nph.15200>
- Pang J, Ryan MH, Siddique KHM, Simpson RJ (2017) Unwrapping the rhizosheath. *Plant Soil* 418:129–139. <https://doi.org/10.1007/s11104-017-3358-y>
- Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat Rev Microbiol* 6:763–775. <https://doi.org/10.1038/nrmicro1987>
- Paterson E, Gebbing T, Abel C, et al (2007) Rhizodeposition shapes rhizosphere microbial community structure in organic soil. *New Phytologist* 173:600–610. <https://doi.org/10.1111/j.1469-8137.2006.01931.x>
- Prashar P, Kapoor N, Sachdeva S (2014) Rhizosphere: its structure, bacterial diversity and significance. *Rev Environ Sci Biotechnol* 13:63–77. <https://doi.org/10.1007/s11157-013-9317-z>
- Richardson AE (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Functional Plant Biol* 28:897. <https://doi.org/10.1071/PP01093>
- 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>

- Rinnan R, Bååth E (2009) Differential Utilization of Carbon Substrates by Bacteria and Fungi in Tundra Soil. *AEM* 75:3611–3620.
<https://doi.org/10.1128/AEM.02865-08>
- Rosling A, Suttle KB, Johansson E, et al (2007) Phosphorous availability influences the dissolution of apatite by soil fungi. *Geobiology* 5:265–280.
<https://doi.org/10.1111/j.1472-4669.2007.00107.x>
- Ryan MH, Tibbett M, Edmonds-Tibbett T, et al (2012) Carbon trading for phosphorus gain: the balance between rhizosphere carboxylates and arbuscular mycorrhizal symbiosis in plant phosphorus acquisition: Carbon trading for phosphorus gain. *Plant, Cell & Environment* 35:2170–2180.
<https://doi.org/10.1111/j.1365-3040.2012.02547.x>
- Ryan P, Delhaize E, Jones D (2001) Function and mechanism of organic anion exudation from plant roots. *Annu Rev Plant Physiol Plant Mol Biol* 52:527–560. <https://doi.org/10.1146/annurev.arplant.52.1.527>
- Sanchez G (2013) *PLS path modeling with R*. Berkeley: Trowchez Editions 383:2013
- Sasse J, Martinoia E, Northen T (2018) Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends in Plant Science* 23:25–41.
<https://doi.org/10.1016/j.tplants.2017.09.003>
- Smith SE, Smith FA (2011) Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales. *Annu Rev Plant Biol* 62:227–250. <https://doi.org/10.1146/annurev-arplant-042110-103846>
- Spaepen S, Vanderleyden J (2011) Auxin and Plant-Microbe Interactions. *Cold Spring Harbor Perspectives in Biology* 3:a001438–a001438.
<https://doi.org/10.1101/cshperspect.a001438>
- Sunita G (2017) Phosphorus Mobilization Strategies of Grain Legumes: An Overview. *Australian Journal of Agricultural Research* 3:1–15

- Sweeney CJ, Vries FT, Dongen BE, Bardgett RD (2021) Root traits explain rhizosphere fungal community composition among temperate grassland plant species. *New Phytol* 229:1492–1507. <https://doi.org/10.1111/nph.16976>
- Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* 1:301–307. [https://doi.org/10.1016/0038-0717\(69\)90012-1](https://doi.org/10.1016/0038-0717(69)90012-1)
- van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Letters* 11:296–310. <https://doi.org/10.1111/j.1461-0248.2007.01139.x>
- Wang Y, Lambers H (2020) Root-released organic anions in response to low phosphorus availability: recent progress, challenges and future perspectives. *Plant Soil* 447:135–156. <https://doi.org/10.1007/s11104-019-03972-8>
- Wang Z, Ma B-L, Gao J, Sun J (2015) Effects of different management systems on root distribution of maize. *Can J Plant Sci* 95:21–28. <https://doi.org/10.4141/cjps-2014-026>
- Wen Z, Li H, Shen Q, et al (2019) Tradeoffs among root morphology, exudation and mycorrhizal symbioses for phosphorus-acquisition strategies of 16 crop species. *New Phytol* 223:882–895. <https://doi.org/10.1111/nph.15833>
- 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>
- White DC, Davis WM, Nickels JS, et al (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* 40:51–62. <https://doi.org/10.1007/BF00388810>
- White PJ, Hammond JP (2008) *The Ecophysiology of Plant-Phosphorus Interactions*. Springer, New York

- Yacoumas A, Honvault N, Houben D, et al (2020) Contrasting Response of Nutrient Acquisition Traits in Wheat Grown on Bisphenol A-Contaminated Soils. *Water Air Soil Pollut* 231:23. <https://doi.org/10.1007/s11270-019-4383-7>
- Yuan J, Zhang N, Huang Q, et al (2015) Organic acids from root exudates of banana help root colonization of PGPR strain *Bacillus amyloliquefaciens* NJN-6. *Sci Rep* 5:13438. <https://doi.org/10.1038/srep13438>
- Zhang J, Wang P, Xue K, et al (2019) Trait complementarity between fine roots of *Stipa purpurea* and their associated arbuscular mycorrhizal fungi along a precipitation gradient in Tibetan alpine steppe. *J Mt Sci* 16:542–547. <https://doi.org/10.1007/s11629-018-5311-9>
- Zhou M, Bai W, Zhang Y, Zhang W-H (2018) Multi-dimensional patterns of variation in root traits among coexisting herbaceous species in temperate steppes. *J Ecol* 106:2320–2331. <https://doi.org/10.1111/1365-2745.12977>