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Abstract

Aims

Plant P acquisition strategies are driven by multiple belowground morphological and physiological traits as well as interactions among these traits. This study aimed to characterize the relationships among traits involved in P acquisition to explore tradeoffs and the main P-acquisition strategies and their mediation by soil type.

Methods

Ten morphological and physiological traits involved in P acquisition were measured across 13 species grown in controlled conditions in two contrasting soils with moderate P limitation.

Results

Tradeoffs between thicker and thinner roots were observed, with thicker roots exhibiting greater carboxylate release or phosphatase activity in the rhizosheath. Tradeoffs and coordination amongst traits were strongly mediated by soil type. Multivariate analysis of functional traits involved in P acquisition highlighted four main P-acquisition strategies relying primarily on morphological traits, physiological traits or a combination thereof.

Conclusions

The diversity of strategies demonstrates a potential for functional diversity benefits in cultivated plant communities via preferential access to different P pools leading to complementarities and reduced competition for resource acquisition. Overall, our results underpin functionally-complementary multispecies crop designs, enhancing P availability and cycling efficiency

Key words: agroecology, cover crops, phosphorus acquisition, root functional trait, tradeoffs

Abbreviations

ΔpH: Change in rhizosheath pH

PME: Phosphomonoesterase activity

RLD: Root length density

SLA: Specific leaf area

SRL: Specific root length

Introduction

Phosphorus (P) is a limiting nutrient in many natural and managed ecosystems due to its strong sorption with soil particles which limits its availability for plants. To overcome low P availability and ensure productive agriculture, P fertilisers are applied, albeit often with very low efficiency (Richardson et al. 2011). In highly-productive fertilized systems, there is a significant gap in P exports and inputs, leading to the accumulation of poorly available organic and inorganic P in the soil (Simpson et al. 2011; Bouwman et al. 2017). In these systems, improving P availability can be achieved by selecting for plant traits and strategies that allow access to the legacy P pool (Menezes-Blackburn et al. 2018). Plants have developed a range of morphological, architectural and physiological traits, granting access to the diverse pools of soil P. Morphological traits such as specific root length (SRL) and architectural traits such as root length density (RLD) allow plants to increase their P-foraging capacity (Pang et al. 2010; Haling et al. 2018; Ma et al. 2018). While only inorganic P in solution is taken up by plants, roots can also mobilize both inorganic and organic P by secreting protons (H^+) to dissolve Ca-phosphate, and carboxylate, which compete with P for binding sites, and thus decrease sorption on mineral surfaces (Wang and Lambers 2019). Plants can also hydrolyze organic P through the release of acid phosphatases, both monoesterases and diesterases (Richardson et al. 2011).

Overall, plant P-acquisition strategies are defined by the expression and association of P-acquisition traits. However, as the expression of these traits has a carbon cost, plants tend to rely mainly on one or a few P-acquisition traits (Pearse et al. 2006; Raven et al. 2018). There may thus be interactions among P-acquisition traits such as root morphological traits being negatively correlated with physiological traits (Zhang et al. 2016; Lyu et al. 2016; Wen et al. 2017).This indicates potential tradeoffs among diverse P-acquisition traits. However, trait interactions and tradeoffs that are central in gaining insight into plant P acquisition, are poorly understood, especially for fast-growing plants such as cover crops (Wen et al. 2019). Understanding tradeoffs and trait-combination effects would allow us to unravel the complexity of the P-acquisition strategies of species and bring new knowledge to design cultivated communities (i.e.multi-species crops) such as cover crops or intercrops to improve P acquisition and availability. Furthermore, trait expression and associated processes are strongly influenced by soil conditions, especially P availability (Raven et al. 2018; Wen et al. 2019). Tradeoffs among life history traits and nutrient-acquisition traits are inconsistent across environmental conditions (Sgrò and Hoffmann 2004; Kong et al. 2019), warranting further investigations as for the consistency of tradeoffs involved in P acquisition.

Intermediate crops, often referred to as cover crops, would offer an opportunity to use a high plant species diversity to enhance P recycling in agroecosystems (Hallama et al. 2019). They can accumulate a large amount of nutrients including P during their growing period (Wendling et al. 2016), which is then released at termination to play a major role in maintaining and improving P availability (Dube et al. 2014; Damon et al. 2014). Moreover, through plant traits associated with a high P-foraging capacity and P mobilization, intermediate crops could acquire P from P pools that are unavailable to the cash crop (Nuruzzaman et al. 2005). A promising opportunity to improve soil P availability for crops can thus be developed by enhancing P cycling in intermediate crops on the basis of their potential to acquire P from poorly-available sources (Richardson et al. 2011). Cover crops present a wide range of P-acquisition traits, therefore potentially presenting different P-acquisition strategies exploiting different P pools (Wendling et al. 2016; Lyu et al. 2016). Examining these strategies and the factors conditioning their expression by characterizing root trait relationships in plant species with diverse root traits would result in a better understanding of P acquisition in crop species and insights for the design of more P-efficient systems. In this study, a greenhouse experiment was carried out to measure morphological, architectural and physiological traits in 13 intermediate crops species with diverse phylogenetic lineages in two contrasting soil types, in order to:

i) Characterize the relationships among traits involved in P acquisition to explore tradeoffs and the main P-acquisition strategies;

ii) Examine whether P forms and soil type mediate expression of tradeoffs and P-acquisition strategies in intermediate crops.

Material and methods

Greenhouse experiment

Soils used in the greenhouse experiment were collected at 5 to 20 cm depth from two fields from north-eastern France, after topsoil (0-5 cm) removal to further decrease P availability. Fields with a known P deficiency were selected to ensure low P availability. A Retisol (formerly called Albeluvisol) and a Calcaric Cambisol (FAO 2014) were selected for their contrasting soil characteristics, mainly their different P forms, in particular their apatite P concentration (Table 1). Prior to use, the soils were dried at ambient temperature, sieved at 2 mm and then mixed with washed sand (22% mass).

Thirteen (sub)species of diverse phylogenetic lineages (Poaceae: *Avena nuda* L., *Avena strigose* Schreb*;* Brassicaceae: *Brassica carinata* A. Braun, *Raphanus sativus* L., *Sinapis alba* L.; Polygonaceae: *Fagopyrum esculentum* Moench.; Fabaceae: *Lens culinaris* Medik., *Pisum sativum* subsp.*arvense* L., *Trifolium alexandrinum* L., *Vicia faba* L., *Vicia sativa* L., and *Vicia villosa* Roth.; Hydrophyllaceae: *Phacelia tanacetifolia* Benth.) were selected for their diverse morphologies, P-acquisition traits and relevance for the local context. The experimental design included two soil types, 13 species and a control (bare soil) with four replicates. Plants were sown mid-January 2018 at two individuals per pot per species in 4.5 L fully filled pots and watered twice a week. Pots were arranged in a random design and greenhouse temperatures were maintained at 22°C during the day (14 hours) and at least 18 °C at night.

Plant traits measurement

At harvest (77 days) plants were manually separated from the bulk soil with special care given to ensure minimum damage. Rhizosheath adhering to the roots up to a maximum of 2 mm after shaking was collected and stored at -20°C for further analysis. Roots were then immersed in 0.20 mM $CaCl_2$ for 1 hour, after removing any remaining soil particles by quickly rinsing with $CaCl_2$. The $CaCl_2$ volume was adjusted to ensure a complete immersion of the root system. The solution was then sampled and stored at -20°C before measuring carboxylic acid exudation by reversed-phase column liquid chromatography (RPLC) (Cawthray 2003; Yacoumas et al. 2020). Briefly, an acid mobile phase (93% 25 mM KH_2PO_4 at pH 2.5 and 7% methanol) allowed a good resolution of five acids (citric, fumaric, maleic, malic, malonic) on a C18 column with a 15 min elution time and a 1 mL min^{-1} flow rate. Total carboxylate release rate was later calculated as the sum of all previously mentioned acids (see Table 2 for abbreviations).

Morphological traits

Aboveground biomass and roots were separated by cutting the stem 1 cm above the first visible root. Roots were then scanned while being immersed in deionised water using an Epson Scanner perfection V800 (Regent Instruments Inc., Québec, Qc, Canada) to produce a 600 dpi image. The image was analysed using WinRHIZO Regular software V.2016a (Regent Instruments Inc., Québec, Qc, Canada) to determine root traits including root surface area (RSA), the percentage of fine root (FR), defined here as length of roots with a diameter < 0.5 mm, and root length density (RLD). After 48 hours drying at 55°C, scanned roots were weighed to calculate specific root length (SRL). Three young but fully-developed leaves per replicate were scanned at 600 dpi, and then dried at 60°C for 48 hours to determine specific leaf area (SLA). All aboveground biomass was dried at 60 °C for 48 hours and weighed.

Nutrient uptake

Dried leaves and stems were digested via acid digestion and a microwave heating treatment (Lange et al. 2016). Between 0.1 and 0.2 g of biomass was introduced in Teflon vessels with 8 mL of 65% (v/v) HNO_3 and 2 mL of 36% (v/v) HCl and heated to 185°C for one hour. Digests were then filtered and P and manganese (Mn) concentrations (as a proxy for rhizosheath carboxylate concentration) determined by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Scientific XSERIES2, Beauvais, France). SRM1573, a certified reference material (tomato leaves) was used as a standard.

Rhizosheath properties

After defrosting, acid phosphomonoesterase activity in the rhizosheath (PME) was measured with a modified buffer at pH 6.5 (Tabatabai and Bremner 1969). Briefly, phosphatase activity was assessed via production of *p*-nitrophenol from sodium *p*-nitrophenyl phosphate during a 1 hour incubation at 37°C with 0.5 g dry soil, 0.2 mL toluene, 4 mL modified buffer and 1 mL substrate. *p*-Nitrophenol release was determined spectrophotometrically at 410 nm after stopping the reaction with 4mL 0.5 M NaOH and 1mL 0.5 M $CaCl_2$ and filtering. Rhizosheath pH was measured on 2 g equivalent dry soil with a 1:10 soil to solution ratio. ΔpH between rhizosheath and bulk soil from unplanted pots post-growth was then calculated.

Data analysis

Linear mixed models (LMM) were used in order to test for differences among plant P uptake and traits among soil types, with species as fixed factors and soil types as random effect. Differences in traits between soils were then investigated with non-parametric tests (Kruskall-Wallis test + post hoc test of Mann Whitney). As multiple differences were observed between soils, further analysis was performed separately for each soil type. To

identify the main covariation in P-acquisition traits and rhizosphere processes, a principal component analysis (PCA) was performed using the "FactomineR" package on log-transformed data (Lê et al. 2008). The number of components was selected to represent more than 75% of the total variability. Tradeoffs between traits were confirmed with Spearman correlation tests as data did not fulfil the condition of normality. Hierarchical classification on principal components (HCPC) was then performed to define groups with similar patterns of P-acquisition traits and influence on the rhizosphere. Species were attached to the group including most of their replicates. A multiresponse permutation procedure (MRPP) confirmed a clear differentiation between clusters in multivariate space. Differences between clusters for each individual factor were then investigated, either with tests of variance (ANOVA and post-hoc test of Tukey) or non-parametric tests (Kruskall-Wallis test + post-hoc test of Mann Whitney).

To investigate the importance of the factors leading to group formation, their influence on P uptake was modelled via generalised linear models (GLM) using the "FactomineR" package. Briefly, all factors were individually tested as predictors of P uptake as well as combinations of the best fitting factors. Models were compared based on second-order Aikake's information (AICc) with the lowest relative value considered the best fit. Differences between models were tested with ANOVA as well as the criteria $\Delta AICc > 2$. Complementary to GLM regression, partial square path modelling (PLS –PM) was performed to underline the relative ability of trait combinations and type for predicting P uptake. Three clusters of variables, or "latent variables" were defined, respectively, the "root morphology" variables encompassing root surface area, SRL, fine root percentage, root length density, the "root physiology" variables encompassing PME activity, carboxylate release and change in rhizosheath pH and the "aboveground traits" variables encompassing SLA, foliar [P] and [Mn]. Verifications were made to ensure model quality, notably unidimensionality of latent variables and cross-loadings between traits associated with a latent variable and other variables as suggested in Sanchez (2013). To ensure the condition of positively correlated variables in a latent variable, the sign of some variables was changed. Variables and components were selected based on their loading and correlation as suggested in Sanchez (2013). Overall model quality was evaluated with the Goodness of Fit (GoF) index. Analyses were performed with the package "plspm" version 0.4.9. All tests were performed in R version 3.6.0 with a significance level of 0.05.

Results

Variation in P-acquisition traits and rhizosphere processes

In the Calcaric Cambisol, the PCA summarised 84% of the total variability, with the first two components representing 50.8% of the total variability (Fig.1). In the Retisol 82% of the total variability was summarised by the PCA, with the first two components representing 51.3% of the total variability (Fig.1). Overall, we observed moderate to strong correlations between morphological indicators such as fine root percentage, RLD, SRL and root surface area (|r| between 0.30 and 0.88). We observed similar patterns between physiological indicators such as change in rhizosheath pH, PME activity and carboxylate release, albeit more differentiated by soil type.

In the Retisol, the first component was formed based on all modifications in the rhizosheath properties measured (pH, PME activity) and carboxylate release, while also presenting a gradient of fine root percentage and root surface area. Aboveground, SLA and leaf P concentration were also important contributors to the first component. The component presented a gradient with strong negative correlations between fine root percentage and PME activity (r=-0.65) and negative correlation between fine root percentage and rhizosheath acidification (r=-0.32). PME activity, rhizosheath acidification and carboxylate release were positively correlated. At the leaf level, SLA was negatively correlated with fine root percentage. The second component was mostly formed based on root physiology and morphology (root length density and surface area Physiological traits important for this axis were carboxylate release as well as to a lesser extent leaf Mn concentration. The component presented a gradient with a negative correlation between carboxylate release and RLD (r=-0.36). Root length density was also positively correlated with root surface area.

In the Calcaric Cambisol, the first component was based on aboveground characteristics such as SLA and foliar Mn concentration. To a lesser extent, the first component was also based on morphological characteristics such as RLD, and rhizosheath modification (rhizosheath acidification). SLA was strongly positively correlated with leaf Mn concentration (r=0.66). Root length density and root surface area were positively correlated. The second axis mostly represented morphological traits such as root surface area, fine root percentage and root length density with a smaller influence of leaf traits such as foliar P concentration and marginal modifications to the rhizosheath such as PME activity. Foliar P concentration was positively correlated with fine root percentage (r=0.33) and SRL (r=0.43), and negatively with root surface area (r=-0.30).

Comparison of P-acquisition traits among different clusters

Based on the PCA scores, we identified four groups via HCPC in the Calcaric Cambisol, five in the Retisol (Fig. 1, Table 3). In the Retisol, the first "physiological/exudation" group L1 (Fig. 1) encompassed lentil, common vetch and white mustard. It showed the greatest exudation of carboxylic acids, significant activity of PME in the rhizosheath, as well as the lowest RLD observed in all groups. The second "intermediate/morphological" group L2 encompassed common and naked oat, Indian mustard, buckwheat and forage radish. It presented a low carboxylate release and PME activity in the rhizosheath, while presenting a small increase in rhizosheath pH and a high RLD, fine root percentage and foliar P concentration compared with other groups. The third "physiological/morphological" group L3 had a strong to intermediate expression of all physiological traits as well as SRL, RLD and SLA and a low foliar P concentration. It grouped forage pea, clover and hairy vetch. The fourth "morphological" group L4, encompassing phacelia, presented low expression of P-mining traits, except for an intermediate carboxylate release, a high fine root percentage and the highest RLD and SRL observed in all groups. Finally the "physiological/mining" group L5, encompassing faba bean, had the lowest SRL and fine root percentage associated with a strong expression of all physiological traits with in particular a strong decrease in rhizosheath pH. Overall the different strategies resulted in similar levels of total P uptake.

In the Calcaric Cambisol, the first "morphological" group C1 was mainly characterised by a high root length density and surface area, an intermediate SRL and a low expression of physiological traits. It encompassed naked oat, forage pea and forage radish. The second "morphological/physiological" group C2, encompassing hairy and common vetch had a significantly higher SLA, stronger rhizosheath acidification, intermediate PME activity in the rhizosheath and higher SRL. The third "intermediate" group C3 encompassed common oat, buckwheat, lentil, phacelia, white mustard and clover. It presented the highest percentage of fine roots, intermediate values of SRL, SLA and the highest foliar P. It also presented the highest value of carboxylate release, while not significantly different from the chemical and morphological groups. Finally the fourth "physiological" group C4, encompassing faba bean, presented the highest PME activity in the rhizosheath, and second-highest carboxylate release. It also presented the lowest fine root percentage and lowest SRL. Overall, these different strategies once again resulted in similar levels of total P uptake.

Modelling trait-combination effects on P acquisition

As we observed a similar P uptake among strategies, we investigated overall relationships between P uptake and factors involved in group formation with GLM (Table 4). PLS PM were also used to highlight the combinations of traits and type of trait best predicting P uptake (Fig. 2). In the Retisol, the best model fit was obtained with

fine root percentage, leaf Mn concentration and PME activity as predictors, while a similar fit was also achieved without PME activity. Fine root percentage was the best predictor when used alone, however, it performed worse than models incorporating multiple traits. While offering a lower fit when incorporating multiples traits, SLA was the second-best predictor, being in itself correlated with multiple morphological and physiological traits. The PLS PM approach (R^2 = 0.5047) produced similar results, with PME activity in the rhizosheath being the best single predictor in the physiological component, followed by rhizosheath acidification, and fine root percentage being the best predictor in the morphological component, followed by root surface area. Similar weights were associated with the physiological (correlation value =0.37) and morphological (correlation value =0.32) components in this soil with a low predicting ability of the aboveground component leading to its removal.

In the Calcaric Cambisol, the best model fit was achieved with the combination of SLA, root surface area, fine root percentage and pH modification in the rhizosheath. Purely morphological models had a significantly poorer fit than models incorporating pH, while rhizosheath acidification alone was a poor predictor. Excluding correlated traits, the best predictors were SLA and root surface area (while not significantly different from SLA+root surface area+change in rhizosheath pH). SLA was the best predictor for single-trait models. PLS PM (R^2 = 0.457) underlined the importance of the morphological component (correlation value =0.31) and aboveground component (correlation value =0.40), while the physiological one was removed due to its poor predicting ability. SLA was the most impactful contributor for the aboveground component. For the morphological component it was root surface area.

Differences among trait variation and tradeoffs per soil type

Trait expressions were significantly different between soil types, with very uneven trait plasticity among species (Table 5). PME activity and carboxylate release were overall significantly lower in Calcaric Cambisol. In contrast, root length density and surface area were higher overall in this soil, especially for Fabaceae and Brassicaceae. Covariation between morphological P-acquisition traits and rhizosphere processes were observed less in Calcaric Cambisol such as the correlation between root surface area and PME and rhizosheath acidification or RLD and carboxylate release observed in Retisol which were not observed in this soil. Positive covariations such as between PME activity in the rhizosheath, carboxylate release and rhizosheath acidification were also not observed in this soil. Similarly, SLA was correlated with change in rhizosheath pH, PME activity and carboxylate release in Retisol, but only with change in rhizosheath pH in Calcaric Cambisol.

Discussion

Tradeoffs in functional traits to understand P acquisition of crop species

Multiple studies have demonstrated the importance of a single trait/few traits for P uptake such as root length density and surface area (Pang et al. 2010; Haling et al. 2018)or PME activity, carboxylate release or rhizosphere acidification (Lambers et al. 2006; Rose et al. 2010; Li et al. 2017; Nobile et al. 2019). However despite increased observations of coordination and tradeoffs among belowground resource-acquisition traits (Roumet et al. 2016; Ma et al. 2018), our understanding of trait interactions and tradeoffs that are central to P acquisition remains limited, especially in crop species (Wen et al. 2019). Our results highlight multiple covariation and tradeoffs between root morphological and physiological traits across a range of fast-growing crop species. Fine root percentage was negatively correlated with PME activity, rhizosheath acidification, and foliar Mn concentration, and marginally with carboxylate release. As foliar Mn can be used as a proxy for rhizosheath carboxylate concentration (Lambers et al. 2015; Pang et al. 2018; Yu et al. 2020), this suggests a greater relevance of physiological strategies associated with P mining for root systems with less fine root percentage. Our results are consistent with recent findings showing that roots with a large diameter present high expression of P-mining traits, while species with thinner fibrous roots express higher levels of morphological traits for P acquisition (Lyu et al. 2016; Wen et al. 2019), extending this observation to a different range of species (intermediate crops species) and context (north-western European soils with moderate P deficiency). Under P stress, plants can modify their root morphology through higher fine root percentage, increased SRL, root hair density and root length density to increase soil foraging at lower cost (Lambers et al. 2006; Shen et al. 2011; Haling et al. 2018). Root physiology can also be modified to increase P availability in the rhizosheath via increased carboxylate, enzyme and proton exudation. Synergistic action of these physiological traits is common, as shown for phytase activity and carboxylate release (Giles et al. 2017) and reinforces the coordination between PME activity in the rhizosheath, carboxylate release and rhizosheath acidification. A possible explanation for this observation is the release of organic P sorbed to soil particles by carboxylates for subsequent mineralisation (Clarholm *et al.*,2015). While both physiological and morphological strategies should benefit P acquisition, tradeoffs between both are suggested to form along a cost / benefit balance due to the important investment they can represent for the plant (Pearse et al. 2006; Raven et al. 2018). Overall our results confirm and extend previous findings in other systems demonstrating multiple coordination and tradeoffs among morphological and physiological traits involved in P acquisition in crop species (Zhang et al. 2016; Giles et al. 2017; Li et al. 2017). Because physiological traits tend to allow greater access to legacy P pools compared with morphological traits,

which allow access to available P pools (Lyu et al. 2016), this morphological/physiological tradeoff offers insight into how to enhance P acquisition and to increase exploitation of the diverse pools of P. Our results demonstrate a potential to select for species at different ends of the tradeoffs spectrum in multispecies systems to increase P acquisition, while reducing competition for resources. Our results constitute a novel contribution toward understanding P acquisition in crop species, and also underline the need for a better understanding of how these trait combinations and tradeoffs are structured in a multi-traits space to form P-acquisition strategies. Such an understanding would offer the opportunity to better design multispecies cropping systems, notably with the main functions of improving P cycling and availability.

Phosphorus-acquisition strategies to design multispecies crops

Plants present very uneven trait plasticity when exposed to different levels of available P (Pearse et al. 2006; Haling et al. 2018; Wen et al. 2019). Uneven plasticity along tradeoffs and covariations suggests both a convergence toward common resource-acquisition strategies and divergence among strategies (Wendling et al. 2016; Li et al. 2017). Plants under P stress rely on resource-acquisitive strategies, either enhancing expression of root morphological traits associated with soil foraging, or modifying their physiological traits to mobilise poorly-available P (Rose et al. 2010; Teng et al. 2013; Lyu et al. 2016). A more complex association of morphological and physiological traits along a cost-benefit axis has also been suggested (Lynch 2015; Weemstra et al. 2016; Wen et al. 2019), prompting further investigations. A certain dichotomy was indeed observed between P-acquisition strategies relying more on physiological or more on morphological traits, corroborating the tradeoffs we observed. However, multivariate analysis highlighted a diversity of intermediate strategies, rather supporting the hypothesis of a complex association of traits along a cost-benefit continuum for P acquisition. Our results offer important new insights into the complex and diverse range of P-acquisition strategies occupying a different trait space, and thus offering opportunities for complementarities in resource acquisition. In both soils, similar extreme strategies could be identified on both ends of a morphological/physiological spectrum. On the morphological end, a strategy could be identified, presenting a low expression of physiological indicators and high root length density/fine roots percentage, mainly encompassing Brassicaceae and Poaceae. This strategy is probably oriented toward soil scavenging, as an important proportion of fine roots can be a way to forage soil at a low cost, while important root length density similarly denotes an important exploration of a given soil volume (Lynch 2015; Yuan et al. 2016; Ma et al. 2018). Similar groupings of species constituting this group (notably Indian mustard, radish, and oat) have been observed before, based on morphological traits, and proposed to be an intermediate strategy between very resource-acquisitive species such as phacelia, and more conservatives ones

such as faba bean (Wendling et al. 2016). On the other end of the spectrum, the several physiological groups, mainly comprising Fabaceae, presented high expressions of all physiological traits with overall lower fine root percentage and SRL. High rhizosheath phosphatase activity, carboxylate release and rhizosheath acidification indicated strategies potentially increasing P availability via mineralisation of organic P, ligand exchange and dissolution of precipitated phosphates, respectively (Hoffland et al. 1989; Jones et al. 2003). While our results confirm the greater effect of Fabaceae on rhizosphere properties compared with other phylogenetic groups (Maltais-Landry 2015), a diverse spectrum of strategies was observed, also within Fabaceae. Two to three clusters per soil expressed intermediate to high values of physiological indicators, and were comprised mainly, but not only of Fabaceae. These groups differed in their expression of morphological traits, especially RLD, fine roots percentage and SRL. This corroborates the existence of a spectrum of P-acquisition strategies combining scavenging and mining processes in intermediate crops. These contrasting strategies, interestingly, did not differ significantly in their P uptake, indicating a potential for maximising P acquisition in multispecies systems via mixing strategies accessing diverse P pools in soils (Lyu et al. 2016; Li et al. 2017) and as such less competition for resources. Such complementarities in resource acquisition among plant species has been suggested as an explanation for the positive effects of functional diversity (Petchey and Gaston 2006; Faucon et al. 2017). Functionally more diverse systems indeed outperform single species for P uptake (Li et al. 2014; Xue et al. 2016). Overall, our results could lead to enhanced P cycling efficiency and P availability in multispecies cropping systems via better designs exploiting more of the total P stock including the pool of legacy P (Richardson et al. 2011). Functional diversity effects on P availability and uptake are not well known however (Huang et al. 2019), and variable shifts in root functional traits and plant P-acquisition strategy should be expected in multispecies conditions (Li et al. 2014). Hence, there is a need for further studies of functional diversity effects on P availability and P cycling. Perspectives include examining functional diversity effect on P uptake and P availability in systems combining different P-acquisition strategies and in different soil and climate contexts. Other traits potentially involved in P acquisition such as root hair length and density (Haling et al. 2013; Yuan et al. 2016), association with mycorrhizal fungi (Smith and Smith 2011; Campos et al. 2018) or rhizosheath bacteria (Richardson et al. 2011) should also be included in future studies to further refine the strategies identified.

Mediation of tradeoffs and P-acquisition strategies according to P forms in soil

Although expression of both P-mining traits and morphological traits are essential for P acquisition, their costs in a specific context may define how plants express and mix their functional traits (Raven et al. 2018; Zhou et al.

2018). Phosphorus-mining traits in particular have proven to have their efficiency strongly impacted by soil type and soil P forms such as proposed for carboxylates in strongly P-sorbing soils (Wang and Lambers 2019). Indeed, we observed greater relevance and coordination of physiological traits in Retisol, while morphological traits were more expressed in Calcaric Cambisol. The modulation of P-acquisition strategies was further evidenced when modelling P uptake via GLM and PLS PM, providing insight into the relative importance of trait type. Phosphorus uptake was best predicted by root morphological traits and aboveground traits in Calcaric Cambisol, and a mixture of root morphological and physiological traits in Retisol. While several single trait correlations with P uptake were previously observed (Pearse et al. 2006; Lambers et al. 2006; Rose et al. 2010; Wen et al. 2017) our results complement our incomplete knowledge of coordination between physiological and morphological responses as well its mediation by soil type and soil P forms (Lyu et al.2016). At low concentration, exudates may not be in a range relevant to significantly impact P uptake in Calcaric Cambisol, or they may have their efficiency impacted by soil characteristics. Adverse effects impacting P mobilisation via carboxylate exudation have indeed been observed, because of "Ca-aided co-adsorption" when released at low concentration, with low amounts of carboxylates increasing adsorption of calcium ions, in turn increasing adsorption of P (Duputel et al. 2013). The higher acid buffering capacity of the Calcaric Cambisol might also have impacted the efficiency of rhizosheath acidification as a P-acquisition trait, further decreasing the potential benefits of physiological traits in this soil. Overall, the strong soil mediation observed offers precious insights into P acquisition. As mentioned, morphological and physiological traits benefit P acquisition from different P pools, offering opportunities for enhanced P cycling in multispecies systems, notably by exploiting the pool of legacy P. However, by reinforcing the importance of plant/soil interactions, our results suggest that improved designs for enhanced P benefits should rely on different processes and strategies in different soils. Some processes such as P mobilisation should be less relevant in some contexts (Maltais-Landry et al. 2014), rather suggesting reliance on a mixture of resource-acquisitive strategies (Wendling et al. 2016). To be able to improve P benefits of intermediate crops, further efforts are needed to investigate the stability and relevance of the tradeoffs and strategies involved in P acquisition for a range of soil types and associated P forms and availability.

Conclusions

Enhancing P cycling and availability in cultivated systems via ecological intensification requires a better understanding of tradeoffs and strategies involved in P acquisition. Morphological and physiological traits comprised diverse P-acquisition strategies structured along an axis of trait covariation and tradeoffs. Our results demonstrate tradeoffs between thicker and thinner roots, exhibiting more physiological traits and morphological

traits, respectively. Reliance on either morphological or physiological traits and combinations thereof for P acquisition were underlined by model approaches, and strongly mediated by soil P forms. The multiple P-acquisition strategies with similar P uptake observed indicates the potential for positive effects of functional diversity via complementarities in resource acquisition, and thus offers insights into mixture designs for improved P benefits. Finally, varied tradeoffs and strategy plasticity amongst soil with different P forms highlighted the importance of considering soil/plant interactions when attempting to improve our understanding of resource-acquisition strategies. Further studies should investigate trait and strategy plasticity across a gradient of functional diversity and various soil properties to help us select for locally optimized crop services.

Authors' Contributions

Nicolas Honvault carried out the experiment and wrote the manuscript with support from Michel-Pierre Faucon, David Houben and Hans Lambers. Stéphane Firmin and Cécile Nobile helped process the data and perform the analyses. All authors contributed to the final version of the manuscript.

References

Bouwman AF, Beusen AHW, Lassaletta L, et al (2017) Lessons from temporal and spatial patterns in global use of N and P fertilizer on cropland. Sci Rep 7:40366. https://doi.org/10.1038/srep40366

Campos P, Borie F, Cornejo P, et al (2018) Phosphorus Acquisition Efficiency Related to Root Traits: IsMycorrhizal 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. J Chromatogr A 1011:233–240. https://doi.org/10.1016/S0021-9673(03)01129-4

Clarholm M, Skyllberg U, Rosling A (2015) Organic acid induced release of nutrients from metal-stabilized soil organic matter – The unbutton model. Soil Biol Biochem 84:168–176. https://doi.org/10.1016/j.soilbio.2015.02.019

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

Dube E, Chiduza C, Muchaonyerwa P (2014) High biomass yielding winter cover crops can improve phosphorus availability in soil. South Afr J Sci 110:1–4. https://doi.org/10.1590/sajs.2014/20130135

Duputel M, Van Hoye F, Toucet J, Gérard F (2013) Citrate adsorption can decrease soluble phosphate concentration in soil: Experimental and modeling evidence. Appl Geochem 39:85–92. https://doi.org/10.1016/j.apgeochem.2013.09.017

FAO (2014) World reference base for soil resources 2014: international soil classification system for naming soils and creating legends for soil maps. FAO, Rome

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

García-Albacete M, Martín A, Cartagena MC (2012) Fractionation of phosphorus biowastes: Characterisation and environmental risk. Waste Manag 32:1061–1068. https://doi.org/10.1016/j.wasman.2012.02.003

Giles CD, George TS, Brown LK, et al (2017) Does the combination of citrate and phytase exudation in Nicotiana tabacum promote the acquisition of endogenous soil organic phosphorus? Plant Soil 412:43–59. https://doi.org/10.1007/s11104-016-2884-3

Haling RE, Brown LK, Bengough AG, et al (2013) Root hairs improve root penetration, root–soil contact, and phosphorus acquisition in soils of different strength. Journal of Experimental Botany 4:3711–3721. https://doi.org/10.1093/jxb/ert200

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

Hoffland E, Findenegg GR, Nelemans JA (1989) Solubilization of rock phosphate by rape. Plant Soil 113:161–165

Huang X, Su J, Li S, et al (2019) Functional diversity drives ecosystem multifunctionality in a Pinus yunnanensis natural secondary forest. Sci Rep 9:6979. https://doi.org/10.1038/s41598-019-43475-1

Jones DL, Dennis PG, Owen AG, van Hees PAW (2003) Organic acid behavior in soils – misconceptions and knowledge gaps. Plant Soil 248:31–41. https://doi.org/10.1023/A:1022304332313

Kong D, Wang J, Wu H, et al (2019) Nonlinearity of root trait relationships and the root economics spectrum. Nat Commun 10:2203. https://doi.org/10.1038/s41467-019-10245-6

Lambers H, Hayes PE, Laliberté E, et al (2015) 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

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

Lê S, Josse J, Husson F (2008) FactoMineR : An *R* Package for Multivariate Analysis. J Stat Softw 25. https://doi.org/10.18637/jss.v025.i01

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

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

Lynch JP (2015) Root phenes that reduce the metabolic costs of soil exploration: opportunities for 21st century agriculture: New roots for agriculture. Plant Cell Environ 38:1775–1784. https://doi.org/10.1111/pce.12451

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

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, 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

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

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

Nuruzzaman M, Lambers H, Bolland MD, Veneklaas EJ (2005) 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

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, Tibbett M, et al (2010) Variation in morphological and physiological parameters in herbaceous perennial legumes in response to phosphorus supply. Plant Soil 331:241–255. https://doi.org/10.1007/s11104-009-0249-x

Pearse SJ, Veneklaas EJ, Cawthray GR, et al (2006) Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. Plant Soil 288:127–139

Petchey OL, Gaston KJ (2006) Functional diversity: back to basics and looking forward. Ecol Lett 9:741–758. https://doi.org/10.1111/j.1461-0248.2006.00924.x

Raven JA, Lambers H, Smith SE, Westoby M (2018) Costs of acquiring phosphorus by vascular land plants: patterns and implications for plant coexistence. New Phytol 217:1420–1427. https://doi.org/10.1111/nph.14967

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

Rose TJ, Hardiputra B, Rengel Z (2010) 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

Roumet C, Birouste M, Picon-Cochard C, et al (2016) Root structure-function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. New Phytol 210:815–826. https://doi.org/10.1111/nph.13828

Sanchez G (2013) PLS path modeling with R. Berkeley Trowchez Ed 383:2013

Sgrò CM, Hoffmann AA (2004) Genetic correlations, tradeoffs and environmental variation. Heredity 93:241–248. https://doi.org/10.1038/sj.hdy.6800532

Shen J, Yuan L, Zhang J, et al (2011) Phosphorus Dynamics: From Soil to Plant. Plant Physiol 156:997–1005. https://doi.org/10.1104/pp.111.175232

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

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

Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1:301–307. https://doi.org/10.1016/0038-0717(69)90012-1

Teng W, Deng Y, Chen X-P, et al (2013) Characterization of root response to phosphorus supply from morphology to gene analysis in field-grown wheat. J Exp Bot 64:1403–1411. https://doi.org/10.1093/jxb/ert023

Wang Y, Lambers H (2019) Root-released organic anions in response to low phosphorus availability: recent progress, challenges and future perspectives. Plant Soil 1–22. https://doi.org/10.1007/s11104-019-03972-8

Weemstra M, Mommer L, Visser EJW, et al (2016) Towards a multidimensional root trait framework: a tree root review. New Phytol 211:1159–1169. https://doi.org/10.1111/nph.14003

Wen Z, Li H, Shen J, Rengel Z (2017) Maize responds to low shoot P concentration by altering root morphology rather than increasing root exudation. Plant Soil 416:377–389. https://doi.org/10.1007/s11104-017-3214-0

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

Xue Y, Xia H, Christie P, et al (2016) Crop acquisition of phosphorus, iron and zinc from soil in cereal/legume intercropping systems: a critical review. Ann Bot 117:363–377. https://doi.org/10.1093/aob/mcv182

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

Yu R-P, Zhang W-P, Yu Y-C, et al (2020) Linking shifts in species composition induced by grazing with root traits for phosphorus acquisition in a typical steppe in Inner Mongolia. Sci Total Environ 712:136495. https://doi.org/10.1016/j.scitotenv.2020.136495

Yuan HM, Blackwell M, Mcgrath S, et al (2016) Morphological responses of wheat (*Triticum aestivum* L.) roots to phosphorus supply in two contrasting soils. J Agric Sci 154:98–108. https://doi.org/10.1017/S0021859615000702

Zhang D, Zhang C, Tang X, et al (2016) Increased soil phosphorus availability induced by faba bean root exudation stimulates root growth and phosphorus uptake in neighbouring maize. New Phytol 209:823–831. https://doi.org/10.1111/nph.13613

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

559 **Table 1** Chemical and physical soil characteristics

	Clay % (<0.002 mm)	Sand % (>0.05 mm)	pH KCl	Ca[a] (g kg^{-1})	N tot (%)	CEC ($cmol_c$ kg^{-1})	Olsen P[c] (mg kg^{-1})	Ca –P[d] (mg kg^{-1})
Calcaric Cambisol	30.7	22.0	7.9	44.7[b]	0.17	9.3	19.8	542
Retisol	22.3	5.4	7.4	2.60	0.10	10.5	16.3	76.7

560 [a]Extractant : Ammonium acetate 0.5 M, EDTA 0.02 M pH 4.65
561 [b]$CaCO_3$ 56.4%
562 [c]According to Olsen (1954)
563 [d]Apatite phosphorus as defined in García-Albacete et al. (2012)

564 **Table 2** List of abbreviations and associated units

Variable	Abbreviation	Unit
Change in rhizosheath pH	ΔpH	-
Fine root percentage (<0.5 mm ⌀)	FR	-
Phosphomonoesterase activity	PME	µg nitrophenol g^{-1} $hour^{-1}$
Root length density	RLD	cm cm^{-3}
Root surface area	RSA	cm^2
Specific leaf area	SLA	mm^2 mg^{-1}
Specific root length	SRL	m g^{-1}
Total carboxylate release rate	TCE	µmol g $root^{-1}$ $hour^{-1}$

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Table 3 Mean values ± standard error (n= 4 to 11) and analyses of variance of phosphorus- acquisition traits and rhizosphere processes per cluster

Soil type	Cluster	TCE	PME	Δ pH	RLD	FR	SRL	SLA	Leaf [P]	Leaf [Mn]
Retisol	L1: Physiological/exudation	41.6 ± 5.3	0.026 ± 0.004	-0.04 ± 0.04	0.09 ± 0.01	0.81 ± 0.02	29.0 ± 4.2	46.1 ± 4.2	2.14 ± 0.19	47 ± 5
	L2: Intermediate/morphological	10.8 ± 1.3	0.017 ± 0.001	0.11 ± 0.04	0.20 ± 0.02	0.85 ± 0.01	14.4 ± 1.6	31.5 ± 2.7	3.20 ± 0.20	166 ± 53
	L3: Physiological/morphological	24.1 ± 4.8	0.036 ± 0.007	-0.16 ± 0.05	0.27 ± 0.03	0.77 ± 0.02	38.2 ± 5.4	62.6 ± 2.6	1.78 ± 0.17	88 ± 9
	L4: Morphological	19.5 ± 2.9	0.020 ± 0.002	0.03 ± 0.09	0.33 ± 0.02	0.87 ± 0.03	65.4 ± 8.8	24.6 ± 4.3	4.17 ± 0.66	36 ± 10
	L5 : Physiological/mining	26.5 ± 5.1	0.038 ± 0.007	-0.29 ± 0.05	0.21 ± 0.02	0.38 ± 0.03	6.8 ± 1.4	60.5 ± 8.8	3.35 ± 0.23	75 ± 4
	p value	<.0001	0.0002[b]	<.0001	<.0001	0.0013[b]	<.0001	<.0001[b]	<.0001	0.0053
Calcaric Cambisol	C1 : Morphological	9.6 ± 2.1	0.011 ± 0.001	-0.05 ± 0.01	0.46 ± 0.02	0.77 ± 0.02	17.3 ± 2.4	31.2 ± 3.9	1.90 ± 0.22	52 ± 9
	C2: Morphological/Physiological	17.0 ± 3.2	0.019 ± 0.004	-0.22 ± 0.02	0.29 ± 0.05	0.72 ± 0.03	35.4 ± 3.5	71.4 ± 7.3	2.16 ± 0.25	125 ± 15
	C3: Intermediate	25.0 ± 7.1	0.010 ± 0.001	-0.07 ± 0.01	0.22 ± 0.02	0.82 ± 0.01	28.9 ± 3.9	40.8 ± 3.9	2.91 ± 0.22	135 ± 15
	C4: Physiological	24.8 ± 13.9	0.022 ± 0.001	-0.05 ± 0.03	0.29 ± 0.05	0.32 ± 0.02	8.1 ± 1.1	57.6 ± 5.1	1.87 ± 0.34	295 ± 34
	p value	0.0169	0.0079[b]	<.0001[b]	<.0001[b]	0.0001[b]	0.0003	0.0002[b]	0.0161	<.0001

[b]:Kruskall-Wallis test; FR: Fine root percentage (<0.5 mm diameter), Leaf [Mn]: Leaf manganese concentration (µg Mn g^{-1}), Leaf [P]: Leaf phosphorus concentration (mg P g^{-1}), PME : Phosphomonoesterase activity (µg nitrophenol g^{-1} $hour^{-1}$), RLD: Root length density (cm cm^{-3}), SLA: Specific leaf area (mm^2 mg^{-1}), SRL: Specific root length (m g^{-1}), TCE: Total carboxylate release rate (µmol g $root^{-1}hour^{-1}$), ΔpH: Change in rhizosheath pH.

570 **Table 4** Selected models fitted to phosphorus uptake

Soil type	Models	AICc	Δ AICc
Retisol	FR + Leaf [Mn] + PME	-18.8	0.0
	FR + Leaf [Mn]	-17.4	1.4
	FR	-16.5	2.3
	SLA	-14.5	4.3
	PME	-12.5	6.2
	Leaf [Mn]	-8.2	10.6
Calcaric Cambisol	FR + ΔpH + SLA + RSA	-43.4	0.0
	ΔpH + SLA + RSA	-39.5	3.9
	FR + SLA + RSA	-39.5	3.9
	SLA	-35.1	8.4
	RSA	-30.7	12.7
	FR	-25.8	17.6
	ΔpH	-23.4	20.0

571 Sorted from best fit to worst. AICc: second order Aikake's information, Δ AICc: Difference between AICc model and lowest AICc observed. FR: Fine root percentage (<0.5
572 mm ⌀), Leaf [Mn]: Leaf manganese concentration, PME: Phosphomonoesterase activity, RSA: Root surface area, SLA: Specific leaf area, ΔpH: change in rhizosheath pH

573 **Table 5** Average trait value ± standard error and analyses of variance per species and per soil

Soil	Species	FR	Leaf [Mn]	Leaf [P]	PME	RLD	RSA	SLA	SRL	TCE	Δ pH
Retisol	*Avena nuda*	0.79±0.02	48±16	3.58±0.10	0.020±0.002	0.14±0.03	60±11	32.9±2.3	10.8±3.1	24.2±15.0	0.19±0.03
	Avena strigosa	0.79±0.02	144±31	2.90±0.17	0.016±0.001	0.27±0.09	103±29	33.0±2.6	14.7±1.9	10.4±2.6	0.01±0.03
	Brassica carinata	0.83±0.02	72±4	2.14±0.19	0.015±0.002	0.18±0.02	70±9	27.4±4.9	13.9±1.4	17.1±2.4	0.01±0.05
	Fagopyrum esculentum	0.88±0.01	456±121	3.79±0.50	0.020±0.001	0.21±0.04	66±12	46.3±5.8	17.2±7.0	6.8±2.3	0.10±0.10
	Lens culinaris	0.78±0.01	47±8	2.52±0.16	0.025±0.001	0.07±0.02	38±11	54.9±2.8	42.8±4.5	48.2±10.1	-0.04±0.09
	Pisum sativum	0.74±0.02	45±10	1.16±0.07	0.032±0.005	0.22±0.07	126±42	66.2±3.1	24.9±7.9	29.5±12.5	-0.13±0.03
	Phacelia tanacetifolia	0.87±0.03	40±12	4.79±0.32	0.020±0.002	0.27±0.07	96±29	25.3±4.0	65.4±8.9	23.7±5.0	-0.06±0.02
	Raphanus sativus	0.68±0.22	36±9	3.05±0.40	0.013±0.001	0.17±0.07	66±15	22.7±3.6	19.5±7.5	13.0±2.4	0.27±0.04
	Sinapis alba	0.9±0.020	34±2	2.04±0.13	0.018±0.001	0.11±0.03	34±5	32.1±7.1	17.5±5.2	25.1±5.4	-0.08±0.05
	Trifolium alexandrinum	0.86±0.02	106±12	2.13±0.22	0.027±0.002	0.23±0.04	92±19	59.1±3.3	48.3±11.1	22.8±2.9	-0.29±0.03
	Vicia faba	0.38±0.02	75±4	3.35±0.23	0.037±0.005	0.21±0.01	191±19	55.2±8.2	6.5±1.1	25.2±3.8	-0.31±0.04
	Vicia sativa	0.79±0.01	64±7.	1.82±0.25	0.053±0.021	0.12±0.03	66±19	54.6±5.4	34.6±9.0	33.0±7.4	-0.04±0.06
	Vicia villosa	0.72±0.02	100±15	1.74±0.24	0.033±0.004	0.32±0.06	183±45	63.8±7.3	38.1±5.3	32.5±13.9	-0.11±0.09
	p value	0.0002[a]	0.0006[a]	<0.0001	0.0026[a]	0.0020	<0.0001	0.0004[a]	<0.0001	0.0126	<0.0001
Calcaric Cambisol	*Avena nuda*	0.74±0.02	33 ±6	1.63±0.28	0.013±0.001	0.47±0.05	247±37	29.67±1.0	9.5±2.9	7.9±4.5	-0.03±0.01
	Avena strigosa	0.82±0.03	148±17	2.69±0.32	0.013±0.001	0.30±0.06	127±35	39.6±2.1	28.7±9.3	13.8±4.6	-0.12±0.03
	Brassica carinata	0.83±0.01	92±31	2.03±0.18	0.008±0.001	0.37±0.03	146±14	21.8±3.4	15.4±1.2	12.0±2.3	-0.01±0.01
	Fagopyrum esculentum	0.84±0.01	67±3	3.56±0.27	0.009±0.001	0.27±0.09	105±38	44.4±8.7	16.1±3.3	6.2±1.9	-0.10±0.01
	Lens culinaris	0.72±0.01	252±30	2.91±0.15	0.013±0.002	0.22±0.06	127±42	58.7±7.4	34.6±4.7	23.1±1.4	-0.09±0.02
	Pisum sativum	0.66±0.01	93±27	0.99±0.22	0.009±0.003	0.34±0.07	223±46	64.9±6.2	24.5±3.1	14.6±3.8	-0.07±0.05
	Phacelia tanacetifolia	0.87±0.01	53±10	3.71±0.40	0.012±0.001	0.19±0.06	67±22	18.6±2.9	53.1±11.0	78.4±32.8	-0.03±0.02
	Raphanus sativus	0.85±0.02	47±12	1.99±0.29	0.012±0.001	0.44±0.06	163±28	17.5±2.3	27.8±2.7	9.9±1.9	-0.07±0.01
	Sinapis alba	0.86±0.01	91±22	1.71±0.33	0.009±0.003	0.12±0.03	44±9	31.8±2.8	10.9±2.1	17.6±3.3	-0.08±0.01
	Trifolium alexandrinum	0.84±0.02	180±30	4.19±0.36	0.010±0.001	0.13±0.02	57±11	60.4±1.8	28.2±8.8	12.8±2.1	-0.10±0.02
	Vicia faba	0.32±0.02	295±34	1.87±0.34	0.022±0.001	0.29±0.05	287±53	57.6±5.1	8.1±1.1	24.8±13.9	-0.05±0.03
	Vicia sativa	0.67±0.04	123±7	2.39±0.27	0.009±0.001	0.30±0.08	198±57	76.0±14.3	32.4±5.7	23.5±5.0	-0.26±0.04
	Vicia villosa	0.74±0.04	141±27	2.51±0.23	0.028±0.008	0.36±0.08	205±49	67.2±10.7	38.8±4.0	14.0±3.6	-0.15±0.02
	p value	<0.0001[a]	<0.0001	<0.0001	0.0123[a]	0.0276[a]	0.0051[a]	<0.0001	<0.0001	0.0169	0.0012[a]

575 [a] Kruskal-Wallis test. Analysis performed on log transformed data. FR: Fine root percentage (<0.5 mm diameter), Leaf [Mn]: Leaf manganese concentration (µg Mn g^{-1}), Leaf
576 [P]: Leaf phosphorus concentration (mg P g^{-1}), PME : Phosphomonoesterase activity (µg nitrophenol g^{-1} $hour^{-1}$), RLD: Root length density (cm cm^{-3}), RSA: Root surface area
577 (cm^2), SLA: Specific leaf area (mm^2 mg^{-1}), SRL: Specific root length (m g^{-1}), TCE: Total carboxylate release rate (µmol g $root^{-1}$ $hour^{-1}$), ΔpH: Change in rhizosheath pH.
578

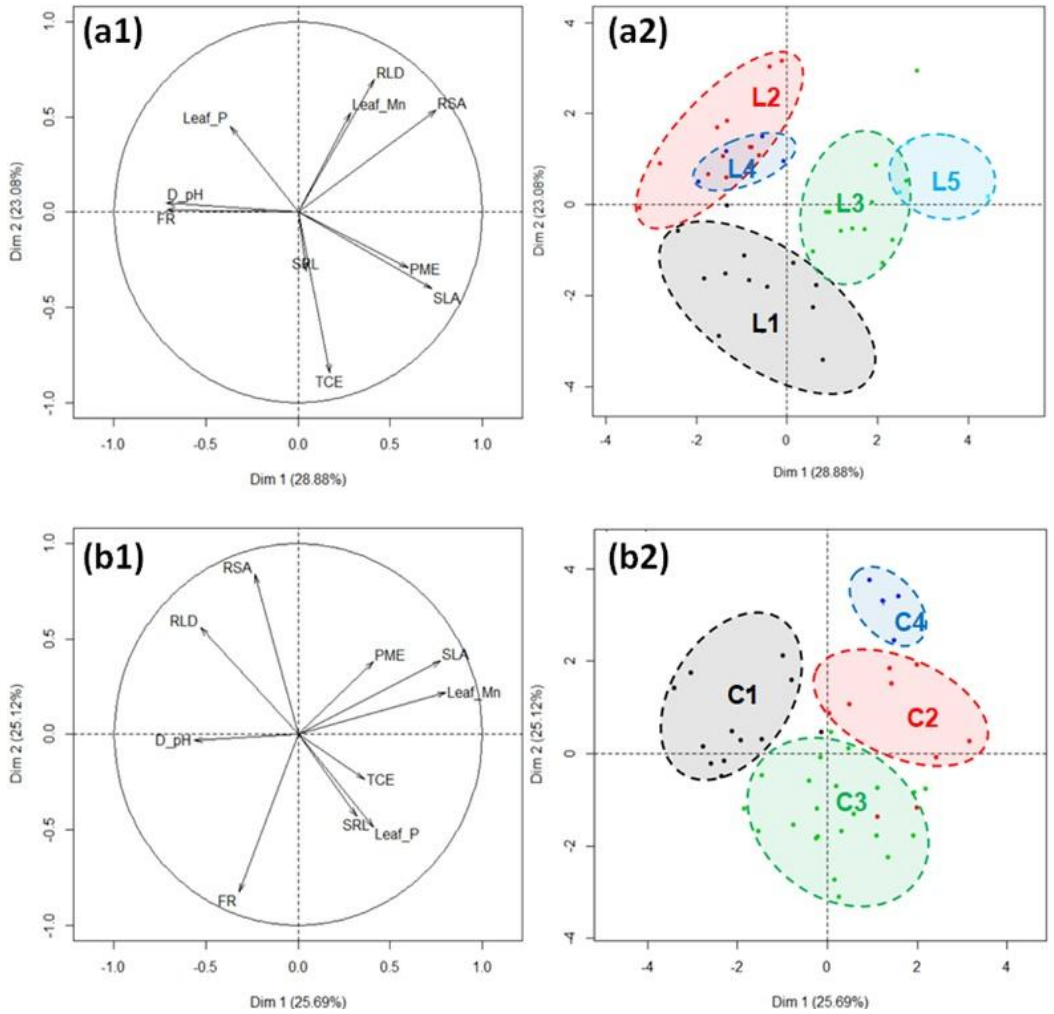

Fig.1 Principal component analysis (PCA) of functional traits and rhizosphere processes involved in phosphorus acquisition in retisol (**a**) and Calcaric Cambisol (**b**)

(1) Variable covariation along first two components, (**2)** clusters formed with Hierarchical Classification on Principal Components (HCPC). Abbreviations: D_pH: change in rhizosheath pH; FR: percentage roots with diameters less than 0.5 mm; Leaf_Mn: foliar manganese concentration; Leaf_P: foliar phosphorus concentration; PME: acid phosphomonoesterase activity; RLD: root length density; RSA: root surface area; SLA: specific leaf area; SRL: specific root area; TCE: total carboxylate release rate.

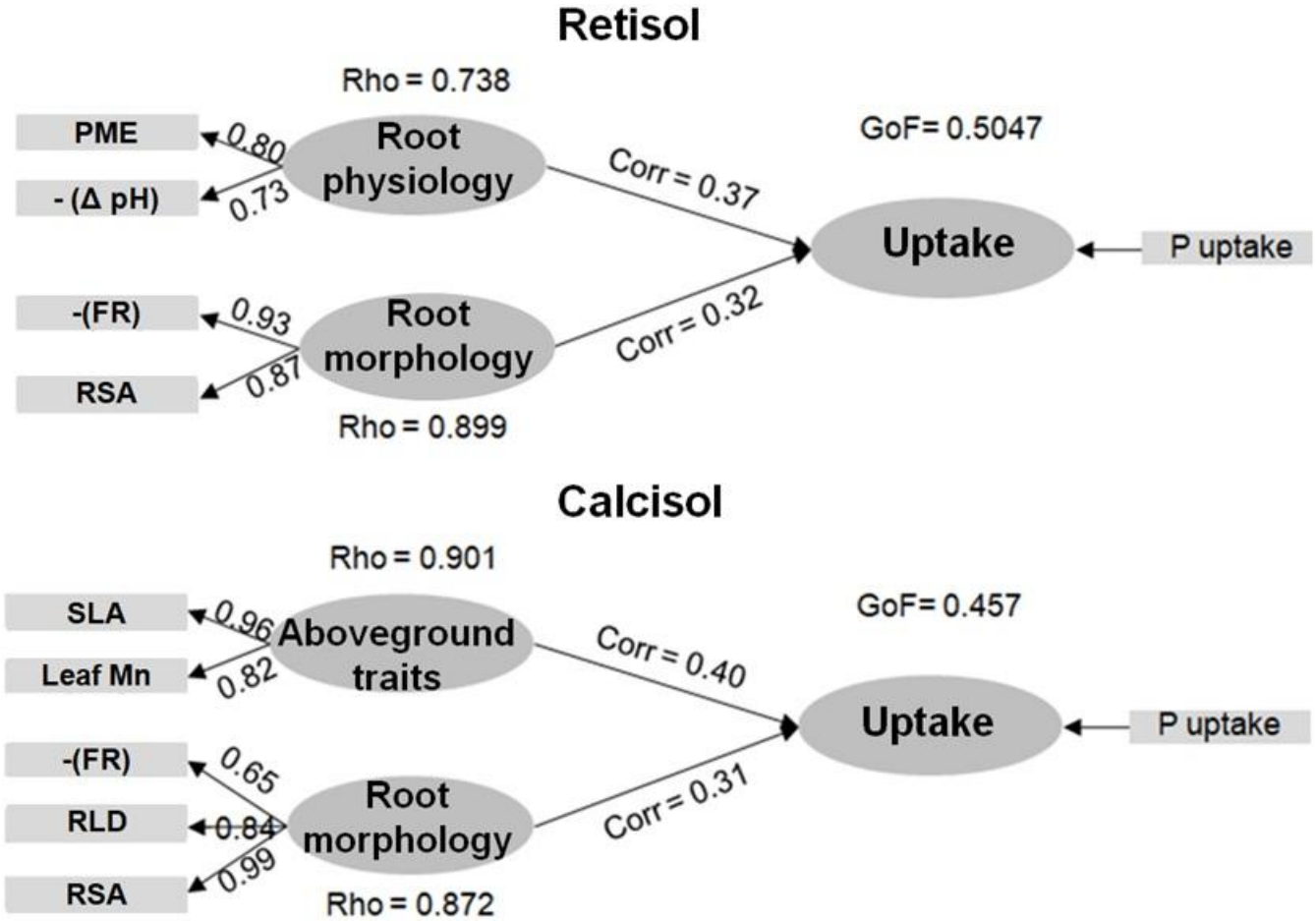

Fig.2 Partial least square- path models predicting phosphorus uptake

Based on three latent variables, namely root physiology, root morphology and aboveground traits in two soils. Corr is the correlation effect, Rho the Dilon-Golstein coefficient. Variables negatively transformed indicated by -(variable). GoF= Goodness of Fit of the model. ΔpH: change in rhizosheath pH; FR: percentage roots with diameters less than 0.5 mm; Leaf Mn: foliar manganese concentration; PME: acid phosphomonoesterase activity; RLD: root length density; RSA: root surface area; SLA: specific leaf area.