



**HAL**  
open science

## Tradeoffs among phosphorus-acquisition root traits of crop species for agroecological intensification

Nicolas Honvault, David Houben, Cécile Nobile, Stéphane Firmin, Hans Lambers, Michel-Pierre Faucon

### ► To cite this version:

Nicolas Honvault, David Houben, Cécile Nobile, Stéphane Firmin, Hans Lambers, et al.. Tradeoffs among phosphorus-acquisition root traits of crop species for agroecological intensification. *Plant and Soil*, 2021, 461, pp.137-150. 10.1007/s11104-020-04584-3 . hal-02860461

**HAL Id: hal-02860461**

**<https://hal.science/hal-02860461>**

Submitted on 8 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Tradeoffs among phosphorus-acquisition root traits of crop species for agroecological**  
2 **intensification**

3 Nicolas Honvault\*<sup>a,b</sup>, David Houben<sup>a</sup>, Cécile Nobile<sup>a</sup>, Stéphane Firmin<sup>a</sup>, Hans Lambers<sup>c</sup>, Michel-Pierre  
4 Faucon\*<sup>a</sup>

5 <sup>a</sup>AGHYLE, UP 2018.C101, SFR Condorcet FR CNRS 3417, UniLaSalle, 60026 Beauvais, France

6 <sup>b</sup>VIVESCIA 2 Rue Clément Ader, 51100 Reims, France

7 <sup>c</sup>School of Biological Sciences and Institute of Agriculture, University of Western Australia

8 \*Corresponding authors: Nicolas Honvault : [nicolas.honvault@unilasalle.fr](mailto:nicolas.honvault@unilasalle.fr) (+33 7 81 48 84 27); Michel-Pierre

9 Faucon : [michel-pierre.faucon@unilasalle.fr](mailto:michel-pierre.faucon@unilasalle.fr)

10 **Acknowledgements**

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

14 **Abstract**

15 Aims

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

19 Methods

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

22 Results

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

27 Conclusions

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

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

33 **Abbreviations**

34  $\Delta$ pH: Change in rhizosheath pH

35 PME: Phosphomonoesterase activity

36 RLD: Root length density

37 SLA: Specific leaf area

38 SRL: Specific root length

## 39 **Introduction**

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

54 Overall, plant P-acquisition strategies are defined by the expression and association of P-acquisition traits.  
55 However, as the expression of these traits has a carbon cost, plants tend to rely mainly on one or a few P-  
56 acquisition traits (Pearse et al. 2006; Raven et al. 2018). There may thus be interactions among P-acquisition  
57 traits such as root morphological traits being negatively correlated with physiological traits (Zhang et al. 2016;  
58 Lyu et al. 2016; Wen et al. 2017). This indicates potential tradeoffs among diverse P-acquisition traits. However,  
59 trait interactions and tradeoffs that are central in gaining insight into plant P acquisition, are poorly understood,  
60 especially for fast-growing plants such as cover crops (Wen et al. 2019). Understanding tradeoffs and trait-  
61 combination effects would allow us to unravel the complexity of the P-acquisition strategies of species and bring  
62 new knowledge to design cultivated communities (i.e. multi-species crops) such as cover crops or intercrops to  
63 improve P acquisition and availability. Furthermore, trait expression and associated processes are strongly  
64 influenced by soil conditions, especially P availability (Raven et al. 2018; Wen et al. 2019). Tradeoffs among life  
65 history traits and nutrient-acquisition traits are inconsistent across environmental conditions (Sgrò and Hoffmann  
66 2004; Kong et al. 2019), warranting further investigations as for the consistency of tradeoffs involved in P  
67 acquisition.

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

- 83 i) Characterize the relationships among traits involved in P acquisition to explore tradeoffs and the  
84 main P-acquisition strategies;
- 85 ii) Examine whether P forms and soil type mediate expression of tradeoffs and P-acquisition  
86 strategies in intermediate crops.

## 87 **Material and methods**

### 88 **Greenhouse experiment**

89 Soils used in the greenhouse experiment were collected at 5 to 20 cm depth from two fields from north-eastern  
90 France, after topsoil (0-5 cm) removal to further decrease P availability. Fields with a known P deficiency were  
91 selected to ensure low P availability. A Retisol (formerly called Albeluvisol) and a Calcaric Cambisol (FAO  
92 2014) were selected for their contrasting soil characteristics, mainly their different P forms, in particular their  
93 apatite P concentration (Table 1). Prior to use, the soils were dried at ambient temperature, sieved at 2 mm and  
94 then mixed with washed sand (22% mass).

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

### 104 **Plant traits measurement**

105 At harvest (77 days) plants were manually separated from the bulk soil with special care given to ensure  
106 minimum damage. Rhizosheath adhering to the roots up to a maximum of 2 mm after shaking was collected and  
107 stored at -20°C for further analysis. Roots were then immersed in 0.20 mM CaCl<sub>2</sub> for 1 hour, after removing any  
108 remaining soil particles by quickly rinsing with CaCl<sub>2</sub>. The CaCl<sub>2</sub> volume was adjusted to ensure a complete  
109 immersion of the root system. The solution was then sampled and stored at -20°C before measuring carboxylic  
110 acid exudation by reversed-phase column liquid chromatography (RPLC) (Cawthray 2003; Yacoumas et al.  
111 2020). Briefly, an acid mobile phase (93% 25 mM KH<sub>2</sub>PO<sub>4</sub> at pH 2.5 and 7% methanol) allowed a good  
112 resolution of five acids (citric, fumaric, maleic, malic, malonic) on a C18 column with a 15 min elution time and  
113 a 1 mL min<sup>-1</sup> flow rate. Total carboxylate release rate was later calculated as the sum of all previously mentioned  
114 acids (see Table 2 for abbreviations).

### 115 **Morphological traits**

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

### 125 **Nutrient uptake**

126 Dried leaves and stems were digested via acid digestion and a microwave heating treatment (Lange et al. 2016).  
127 Between 0.1 and 0.2 g of biomass was introduced in Teflon vessels with 8 mL of 65% (v/v) HNO<sub>3</sub> and 2 mL of  
128 36% (v/v) HCl and heated to 185°C for one hour. Digests were then filtered and P and manganese (Mn)  
129 concentrations (as a proxy for rhizosheath carboxylate concentration) determined by inductively coupled plasma  
130 mass spectrometry (ICP-MS) (Thermo Scientific XSERIES2, Beauvais, France). SRM1573, a certified reference  
131 material (tomato leaves) was used as a standard.

### 132 **Rhizosheath properties**

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

### 140 **Data analysis**

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



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

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

## 171 **Results**

### 172 **Variation in P-acquisition traits and rhizosphere processes**

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

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

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

### 198 **Comparison of P-acquisition traits among different clusters**

199 Based on the PCA scores, we identified four groups via HCPC in the Calcaric Cambisol, five in the Retisol (Fig.  
200 1, Table 3). In the Retisol, the first “physiological/exudation” group L1 (Fig. 1) encompassed lentil, common  
201 vetch and white mustard. It showed the greatest exudation of carboxylic acids, significant activity of PME in the  
202 rhizosheath, as well as the lowest RLD observed in all groups. The second “intermediate/morphological” group  
203 L2 encompassed common and naked oat, Indian mustard, buckwheat and forage radish. It presented a low  
204 carboxylate release and PME activity in the rhizosheath, while presenting a small increase in rhizosheath pH and  
205 a high RLD, fine root percentage and foliar P concentration compared with other groups. The third  
206 “physiological/morphological” group L3 had a strong to intermediate expression of all physiological traits as  
207 well as SRL, RLD and SLA and a low foliar P concentration. It grouped forage pea, clover and hairy vetch. The  
208 fourth “morphological” group L4, encompassing phacelia, presented low expression of P-mining traits, except  
209 for an intermediate carboxylate release, a high fine root percentage and the highest RLD and SRL observed in all  
210 groups. Finally the “physiological/mining” group L5, encompassing faba bean, had the lowest SRL and fine root  
211 percentage associated with a strong expression of all physiological traits with in particular a strong decrease in  
212 rhizosheath pH. Overall the different strategies resulted in similar levels of total P uptake.

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

#### 224 **Modelling trait-combination effects on P acquisition**

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

228 fine root percentage, leaf Mn concentration and PME activity as predictors, while a similar fit was also achieved  
229 without PME activity. Fine root percentage was the best predictor when used alone, however, it performed worse  
230 than models incorporating multiple traits. While offering a lower fit when incorporating multiples traits, SLA  
231 was the second-best predictor, being in itself correlated with multiple morphological and physiological traits.  
232 The PLS PM approach ( $R^2 = 0.5047$ ) produced similar results, with PME activity in the rhizosheath being the  
233 best single predictor in the physiological component, followed by rhizosheath acidification, and fine root  
234 percentage being the best predictor in the morphological component, followed by root surface area. Similar  
235 weights were associated with the physiological (correlation value =0.37) and morphological (correlation value  
236 =0.32) components in this soil with a low predicting ability of the aboveground component leading to its  
237 removal.

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

#### 247 **Differences among trait variation and tradeoffs per soil type**

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

## 257 **Discussion**

### 258 **Tradeoffs in functional traits to understand P acquisition of crop species**

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

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

### 295 **Phosphorus-acquisition strategies to design multispecies crops**

296 Plants present very uneven trait plasticity when exposed to different levels of available P (Pearse et al. 2006;  
297 Haling et al. 2018; Wen et al. 2019). Uneven plasticity along tradeoffs and covariations suggests both a  
298 convergence toward common resource-acquisition strategies and divergence among strategies (Wendling et al.  
299 2016; Li et al. 2017). Plants under P stress rely on resource-acquisitive strategies, either enhancing expression of  
300 root morphological traits associated with soil foraging, or modifying their physiological traits to mobilise poorly-  
301 available P (Rose et al. 2010; Teng et al. 2013; Lyu et al. 2016). A more complex association of morphological  
302 and physiological traits along a cost-benefit axis has also been suggested (Lynch 2015; Weemstra et al. 2016;  
303 Wen et al. 2019), prompting further investigations. A certain dichotomy was indeed observed between P-  
304 acquisition strategies relying more on physiological or more on morphological traits, corroborating the tradeoffs  
305 we observed. However, multivariate analysis highlighted a diversity of intermediate strategies, rather supporting  
306 the hypothesis of a complex association of traits along a cost-benefit continuum for P acquisition. Our results  
307 offer important new insights into the complex and diverse range of P-acquisition strategies occupying a different  
308 trait space, and thus offering opportunities for complementarities in resource acquisition. In both soils, similar  
309 extreme strategies could be identified on both ends of a morphological/physiological spectrum. On the  
310 morphological end, a strategy could be identified, presenting a low expression of physiological indicators and  
311 high root length density/fine roots percentage, mainly encompassing Brassicaceae and Poaceae. This strategy is  
312 probably oriented toward soil scavenging, as an important proportion of fine roots can be a way to forage soil at  
313 a low cost, while important root length density similarly denotes an important exploration of a given soil volume  
314 (Lynch 2015; Yuan et al. 2016; Ma et al. 2018). Similar groupings of species constituting this group (notably  
315 Indian mustard, radish, and oat) have been observed before, based on morphological traits, and proposed to be an  
316 intermediate strategy between very resource-acquisitive species such as phacelia, and more conservatives ones

317 such as faba bean (Wendling et al. 2016). On the other end of the spectrum, the several physiological groups,  
318 mainly comprising Fabaceae, presented high expressions of all physiological traits with overall lower fine root  
319 percentage and SRL. High rhizosheath phosphatase activity, carboxylate release and rhizosheath acidification  
320 indicated strategies potentially increasing P availability via mineralisation of organic P, ligand exchange and  
321 dissolution of precipitated phosphates, respectively (Hoffland et al. 1989; Jones et al. 2003). While our results  
322 confirm the greater effect of Fabaceae on rhizosphere properties compared with other phylogenetic groups  
323 (Maltais-Landry 2015), a diverse spectrum of strategies was observed, also within Fabaceae. Two to three  
324 clusters per soil expressed intermediate to high values of physiological indicators, and were comprised mainly,  
325 but not only of Fabaceae. These groups differed in their expression of morphological traits, especially RLD, fine  
326 roots percentage and SRL. This corroborates the existence of a spectrum of P-acquisition strategies combining  
327 scavenging and mining processes in intermediate crops. These contrasting strategies, interestingly, did not differ  
328 significantly in their P uptake, indicating a potential for maximising P acquisition in multispecies systems via  
329 mixing strategies accessing diverse P pools in soils (Lyu et al. 2016; Li et al. 2017) and as such less competition  
330 for resources. Such complementarities in resource acquisition among plant species has been suggested as an  
331 explanation for the positive effects of functional diversity (Petchey and Gaston 2006; Faucon et al. 2017).  
332 Functionally more diverse systems indeed outperform single species for P uptake (Li et al. 2014; Xue et al.  
333 2016). Overall, our results could lead to enhanced P cycling efficiency and P availability in multispecies  
334 cropping systems via better designs exploiting more of the total P stock including the pool of legacy P  
335 (Richardson et al. 2011). Functional diversity effects on P availability and uptake are not well known however  
336 (Huang et al. 2019), and variable shifts in root functional traits and plant P-acquisition strategy should be  
337 expected in multispecies conditions (Li et al. 2014). Hence, there is a need for further studies of functional  
338 diversity effects on P availability and P cycling. Perspectives include examining functional diversity effect on P  
339 uptake and P availability in systems combining different P-acquisition strategies and in different soil and climate  
340 contexts. Other traits potentially involved in P acquisition such as root hair length and density (Haling et al.  
341 2013; Yuan et al. 2016), association with mycorrhizal fungi (Smith and Smith 2011; Campos et al. 2018) or  
342 rhizosheath bacteria (Richardson et al. 2011) should also be included in future studies to further refine the  
343 strategies identified.

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

345 Although expression of both P-mining traits and morphological traits are essential for P acquisition, their costs in  
346 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 rhizosphere 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.

## 372 **Conclusions**

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



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

385 **Authors' Contributions**

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

389 **References**

- 390 Bouwman AF, Beusen AHW, Lassaletta L, et al (2017) Lessons from temporal and spatial  
391 patterns in global use of N and P fertilizer on cropland. *Sci Rep* 7:40366.  
392 <https://doi.org/10.1038/srep40366>
- 393 Campos P, Borie F, Cornejo P, et al (2018) Phosphorus Acquisition Efficiency Related to  
394 Root Traits: Is Mycorrhizal Symbiosis a Key Factor to Wheat and Barley Cropping?  
395 *Front Plant Sci* 9:752. <https://doi.org/10.3389/fpls.2018.00752>
- 396 Cawthray GR (2003) An improved reversed-phase liquid chromatographic method for the  
397 analysis of low-molecular mass organic acids in plant root exudates. *J Chromatogr A*  
398 1011:233–240. [https://doi.org/10.1016/S0021-9673\(03\)01129-4](https://doi.org/10.1016/S0021-9673(03)01129-4)
- 399 Clarholm M, Skjellberg U, Rosling A (2015) Organic acid induced release of nutrients from  
400 metal-stabilized soil organic matter – The unbutton model. *Soil Biol Biochem* 84:168–  
401 176. <https://doi.org/10.1016/j.soilbio.2015.02.019>
- 402 Damon PM, Bowden B, Rose T, Rengel Z (2014) Crop residue contributions to phosphorus  
403 pools in agricultural soils: A review. *Soil Biol Biochem* 74:127–137.  
404 <https://doi.org/10.1016/j.soilbio.2014.03.003>
- 405 Dube E, Chiduza C, Muchaonyerwa P (2014) High biomass yielding winter cover crops can  
406 improve phosphorus availability in soil. *South Afr J Sci* 110:1–4.  
407 <https://doi.org/10.1590/sajs.2014/20130135>
- 408 Duputel M, Van Hoyer F, Toucet J, Gérard F (2013) Citrate adsorption can decrease soluble  
409 phosphate concentration in soil: Experimental and modeling evidence. *Appl Geochem*  
410 39:85–92. <https://doi.org/10.1016/j.apgeochem.2013.09.017>
- 411 FAO (2014) World reference base for soil resources 2014: international soil classification  
412 system for naming soils and creating legends for soil maps. FAO, Rome
- 413 Faucon M-P, Houben D, Lambers H (2017) Plant Functional Traits: Soil and Ecosystem  
414 Services. *Trends Plant Sci* 22:385–394. <https://doi.org/10.1016/j.tplants.2017.01.005>
- 415 García-Albacete M, Martín A, Cartagena MC (2012) Fractionation of phosphorus biowastes:  
416 Characterisation and environmental risk. *Waste Manag* 32:1061–1068.  
417 <https://doi.org/10.1016/j.wasman.2012.02.003>
- 418 Giles CD, George TS, Brown LK, et al (2017) Does the combination of citrate and phytase  
419 exudation in *Nicotiana tabacum* promote the acquisition of endogenous soil organic  
420 phosphorus? *Plant Soil* 412:43–59. <https://doi.org/10.1007/s11104-016-2884-3>
- 421 Haling RE, Brown LK, Bengough AG, et al (2013) Root hairs improve root penetration, root–  
422 soil contact, and phosphorus acquisition in soils of different strength. *Journal of*  
423 *Experimental Botany* 4:3711–3721. <https://doi.org/10.1093/jxb/ert200>
- 424 Haling RE, Brown LK, Stefanski A, et al (2018) Differences in nutrient foraging among  
425 *Trifolium subterraneum* cultivars deliver improved P-acquisition efficiency. *Plant Soil*  
426 424:539–554. <https://doi.org/10.1007/s11104-017-3511-7>

- 427 Hallama M, Pekrun C, Lambers H, Kandeler E (2019) Hidden miners – the roles of cover  
428 crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant*  
429 *Soil* 434:7–45. <https://doi.org/10.1007/s11104-018-3810-7>
- 430 Hoffland E, Findenegg GR, Nelemans JA (1989) Solubilization of rock phosphate by rape.  
431 *Plant Soil* 113:161–165
- 432 Huang X, Su J, Li S, et al (2019) Functional diversity drives ecosystem multifunctionality in a  
433 *Pinus yunnanensis* natural secondary forest. *Sci Rep* 9:6979.  
434 <https://doi.org/10.1038/s41598-019-43475-1>
- 435 Jones DL, Dennis PG, Owen AG, van Hees PAW (2003) Organic acid behavior in soils –  
436 misconceptions and knowledge gaps. *Plant Soil* 248:31–41.  
437 <https://doi.org/10.1023/A:1022304332313>
- 438 Kong D, Wang J, Wu H, et al (2019) Nonlinearity of root trait relationships and the root  
439 economics spectrum. *Nat Commun* 10:2203. [https://doi.org/10.1038/s41467-019-](https://doi.org/10.1038/s41467-019-10245-6)  
440 [10245-6](https://doi.org/10.1038/s41467-019-10245-6)
- 441 Lambers H, Hayes PE, Laliberté E, et al (2015) Leaf manganese accumulation and  
442 phosphorus-acquisition efficiency. *Trends Plant Sci* 20:83–90.  
443 <https://doi.org/10.1016/j.tplants.2014.10.007>
- 444 Lambers H, Shane MW, Cramer MD, et al (2006) Root Structure and Functioning for  
445 Efficient Acquisition of Phosphorus: Matching Morphological and Physiological  
446 Traits. *Ann Bot* 98:693–713. <https://doi.org/10.1093/aob/mcl114>
- 447 Lange B, Pourret O, Meerts P, et al (2016) Copper and cobalt mobility in soil and  
448 accumulation in a metallophyte as influenced by experimental manipulation of soil  
449 chemical factors. *Chemosphere* 146:75–84.  
450 <https://doi.org/10.1016/j.chemosphere.2015.11.105>
- 451 Lê S, Josse J, Husson F (2008) FactoMineR : An R Package for Multivariate Analysis. *J Stat*  
452 *Softw* 25. <https://doi.org/10.18637/jss.v025.i01>
- 453 Li H, Liu B, McCormack ML, et al (2017) Diverse belowground resource strategies underlie  
454 plant species coexistence and spatial distribution in three grasslands along a  
455 precipitation gradient. *New Phytol* 216:1140–1150. <https://doi.org/10.1111/nph.14710>
- 456 Li L, Tilman D, Lambers H, Zhang F-S (2014) Plant diversity and overyielding: insights from  
457 belowground facilitation of intercropping in agriculture. *New Phytol* 203:63–69.  
458 <https://doi.org/10.1111/nph.12778>
- 459 Lynch JP (2015) Root phenes that reduce the metabolic costs of soil exploration:  
460 opportunities for 21st century agriculture: New roots for agriculture. *Plant Cell*  
461 *Environ* 38:1775–1784. <https://doi.org/10.1111/pce.12451>
- 462 Lyu Y, Tang H, Li H, et al (2016) Major Crop Species Show Differential Balance between  
463 Root Morphological and Physiological Responses to Variable Phosphorus Supply.  
464 *Front Plant Sci* 7:15. <https://doi.org/10.3389/fpls.2016.01939>

- 465 Ma Z, Guo D, Xu X, et al (2018) Evolutionary history resolves global organization of root  
466 functional traits. *Nature* 555:94–97. <https://doi.org/10.1038/nature25783>
- 467 Maltais-Landry G (2015) Legumes have a greater effect on rhizosphere properties (pH,  
468 organic acids and enzyme activity) but a smaller impact on soil P compared to other  
469 cover crops. *Plant Soil* 394:139–154. <https://doi.org/10.1007/s11104-015-2518-1>
- 470 Maltais-Landry G, Scow K, Brennan E (2014) Soil phosphorus mobilization in the  
471 rhizosphere of cover crops has little effect on phosphorus cycling in California  
472 agricultural soils. *Soil Biol Biochem* 78:255–262.  
473 <https://doi.org/10.1016/j.soilbio.2014.08.013>
- 474 Menezes-Blackburn D, Giles C, Darch T, et al (2018) Opportunities for mobilizing  
475 recalcitrant phosphorus from agricultural soils: a review. *Plant Soil* 427:5–16.  
476 <https://doi.org/10.1007/s11104-017-3362-2>
- 477 Nobile C, Houben D, Michel E, et al (2019) Phosphorus-acquisition strategies of canola,  
478 wheat and barley in soil amended with sewage sludges. *Sci Rep* 9:14878.  
479 <https://doi.org/10.1038/s41598-019-51204>
- 480 Nuruzzaman M, Lambers H, Bolland MD, Veneklaas EJ (2005) Phosphorus benefits of  
481 different legume crops to subsequent wheat grown in different soils of Western  
482 Australia. *Plant Soil* 271:175–187. <https://doi.org/10.1007/s11104-004-2386-6>
- 483 Olsen SR (1954) Estimation of Available Phosphorus in Soils by Extraction with Sodium  
484 Bicarbonate. U.S. Department of Agriculture
- 485 Pang J, Bansal R, Zhao H, et al (2018) The carboxylate-releasing phosphorus-mobilizing  
486 strategy can be proxied by foliar manganese concentration in a large set of chickpea  
487 germplasm under low phosphorus supply. *New Phytol* 219:518–529.  
488 <https://doi.org/10.1111/nph.15200>
- 489 Pang J, Ryan MH, Tibbett M, et al (2010) Variation in morphological and physiological  
490 parameters in herbaceous perennial legumes in response to phosphorus supply. *Plant*  
491 *Soil* 331:241–255. <https://doi.org/10.1007/s11104-009-0249-x>
- 492 Pearse SJ, Veneklaas EJ, Cawthray GR, et al (2006) Carboxylate release of wheat, canola and  
493 11 grain legume species as affected by phosphorus status. *Plant Soil* 288:127–139
- 494 Petchey OL, Gaston KJ (2006) Functional diversity: back to basics and looking forward. *Ecol*  
495 *Lett* 9:741–758. <https://doi.org/10.1111/j.1461-0248.2006.00924.x>
- 496 Raven JA, Lambers H, Smith SE, Westoby M (2018) Costs of acquiring phosphorus by  
497 vascular land plants: patterns and implications for plant coexistence. *New Phytol*  
498 217:1420–1427. <https://doi.org/10.1111/nph.14967>
- 499 Richardson AE, Lynch JP, Ryan PR, et al (2011) Plant and microbial strategies to improve the  
500 phosphorus efficiency of agriculture. *Plant Soil* 349:121–156.  
501 <https://doi.org/10.1007/s11104-011-0950-4>

- 502 Rose TJ, Hardiputra B, Rengel Z (2010) Wheat, canola and grain legume access to soil  
503 phosphorus fractions differs in soils with contrasting phosphorus dynamics. *Plant Soil*  
504 326:159–170. <https://doi.org/10.1007/s11104-009-9990-4>
- 505 Roumet C, Birouste M, Picon-Cochard C, et al (2016) Root structure-function relationships in  
506 74 species: evidence of a root economics spectrum related to carbon economy. *New*  
507 *Phytol* 210:815–826. <https://doi.org/10.1111/nph.13828>
- 508 Sanchez G (2013) PLS path modeling with R. Berkeley Trowchez Ed 383:2013
- 509 Sgrò CM, Hoffmann AA (2004) Genetic correlations, tradeoffs and environmental variation.  
510 *Heredity* 93:241–248. <https://doi.org/10.1038/sj.hdy.6800532>
- 511 Shen J, Yuan L, Zhang J, et al (2011) Phosphorus Dynamics: From Soil to Plant. *Plant*  
512 *Physiol* 156:997–1005. <https://doi.org/10.1104/pp.111.175232>
- 513 Simpson RJ, Oberson A, Culvenor RA, et al (2011) Strategies and agronomic interventions to  
514 improve the phosphorus-use efficiency of farming systems. *Plant Soil* 349:89–120.  
515 <https://doi.org/10.1007/s11104-011-0880-1>
- 516 Smith SE, Smith FA (2011) Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth:  
517 New Paradigms from Cellular to Ecosystem Scales. *Annu Rev Plant Biol* 62:227–250.  
518 <https://doi.org/10.1146/annurev-arplant-042110-103846>
- 519 Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil  
520 phosphatase activity. *Soil Biol Biochem* 1:301–307. [https://doi.org/10.1016/0038-](https://doi.org/10.1016/0038-0717(69)90012-1)  
521 [0717\(69\)90012-1](https://doi.org/10.1016/0038-0717(69)90012-1)
- 522 Teng W, Deng Y, Chen X-P, et al (2013) Characterization of root response to phosphorus  
523 supply from morphology to gene analysis in field-grown wheat. *J Exp Bot* 64:1403–  
524 1411. <https://doi.org/10.1093/jxb/ert023>
- 525 Wang Y, Lambers H (2019) Root-released organic anions in response to low phosphorus  
526 availability: recent progress, challenges and future perspectives. *Plant Soil* 1–22.  
527 <https://doi.org/10.1007/s11104-019-03972-8>
- 528 Weemstra M, Mommer L, Visser EJW, et al (2016) Towards a multidimensional root trait  
529 framework: a tree root review. *New Phytol* 211:1159–1169.  
530 <https://doi.org/10.1111/nph.14003>
- 531 Wen Z, Li H, Shen J, Rengel Z (2017) Maize responds to low shoot P concentration by  
532 altering root morphology rather than increasing root exudation. *Plant Soil* 416:377–  
533 389. <https://doi.org/10.1007/s11104-017-3214-0>
- 534 Wen Z, Li H, Shen Q, et al (2019) Tradeoffs among root morphology, exudation and  
535 mycorrhizal symbioses for phosphorus- acquisition strategies of 16 crop species. *New*  
536 *Phytol* 223:882–895. <https://doi.org/10.1111/nph.15833>
- 537 Wendling M, Büchi L, Amossé C, et al (2016) Influence of root and leaf traits on the uptake  
538 of nutrients in cover crops. *Plant Soil* 409:419–434. [https://doi.org/10.1007/s11104-](https://doi.org/10.1007/s11104-016-2974-2)  
539 [016-2974-2](https://doi.org/10.1007/s11104-016-2974-2)

- 540 Xue Y, Xia H, Christie P, et al (2016) Crop acquisition of phosphorus, iron and zinc from soil  
541 in cereal/legume intercropping systems: a critical review. *Ann Bot* 117:363–377.  
542 <https://doi.org/10.1093/aob/mcv182>
- 543 Yacoumas A, Honvault N, Houben D, et al (2020) Contrasting Response of Nutrient  
544 Acquisition Traits in Wheat Grown on Bisphenol A-Contaminated Soils. *Water Air  
545 Soil Pollut* 231:23. <https://doi.org/10.1007/s11270-019-4383-7>
- 546 Yu R-P, Zhang W-P, Yu Y-C, et al (2020) Linking shifts in species composition induced by  
547 grazing with root traits for phosphorus acquisition in a typical steppe in Inner  
548 Mongolia. *Sci Total Environ* 712:136495.  
549 <https://doi.org/10.1016/j.scitotenv.2020.136495>
- 550 Yuan HM, Blackwell M, Mcgrath S, et al (2016) Morphological responses of wheat (  
551 *Triticum aestivum* L.) roots to phosphorus supply in two contrasting soils. *J Agric Sci*  
552 154:98–108. <https://doi.org/10.1017/S0021859615000702>
- 553 Zhang D, Zhang C, Tang X, et al (2016) Increased soil phosphorus availability induced by  
554 faba bean root exudation stimulates root growth and phosphorus uptake in  
555 neighbouring maize. *New Phytol* 209:823–831. <https://doi.org/10.1111/nph.13613>
- 556 Zhou M, Bai W, Zhang Y, Zhang W-H (2018) Multi-dimensional patterns of variation in root  
557 traits among coexisting herbaceous species in temperate steppes. *J Ecol* 106:2320–  
558 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 <sup>a</sup> (g kg <sup>-1</sup> )	N tot (%)	CEC (cmol <sub>c</sub> kg <sup>-1</sup> )	Olsen P <sup>c</sup> (mg kg <sup>-1</sup> )	Ca -P <sup>d</sup> (mg kg <sup>-1</sup> )
Calcaric	30.7	22.0	7.9	44.7 <sup>b</sup>	0.17	9.3	19.8	542
Cambisol								
Retisol	22.3	5.4	7.4	2.60	0.10	10.5	16.3	76.7

560 <sup>a</sup>Extractant : Ammonium acetate 0.5 M, EDTA 0.02 M pH 4.65

561 <sup>b</sup>CaCO<sub>3</sub> 56.4%

562 <sup>c</sup>According to Olsen (1954)

563 <sup>d</sup>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	$\Delta$ pH	-
Fine root percentage (<0.5 mm $\varnothing$ )	FR	-
Phosphomonoesterase activity	PME	$\mu\text{g nitrophenol g}^{-1} \text{ hour}^{-1}$
Root length density	RLD	$\text{cm cm}^{-3}$
Root surface area	RSA	$\text{cm}^2$
Specific leaf area	SLA	$\text{mm}^2 \text{ mg}^{-1}$
Specific root length	SRL	$\text{m g}^{-1}$
Total carboxylate release rate	TCE	$\mu\text{mol g root}^{-1} \text{ hour}^{-1}$

565

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

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

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

570

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

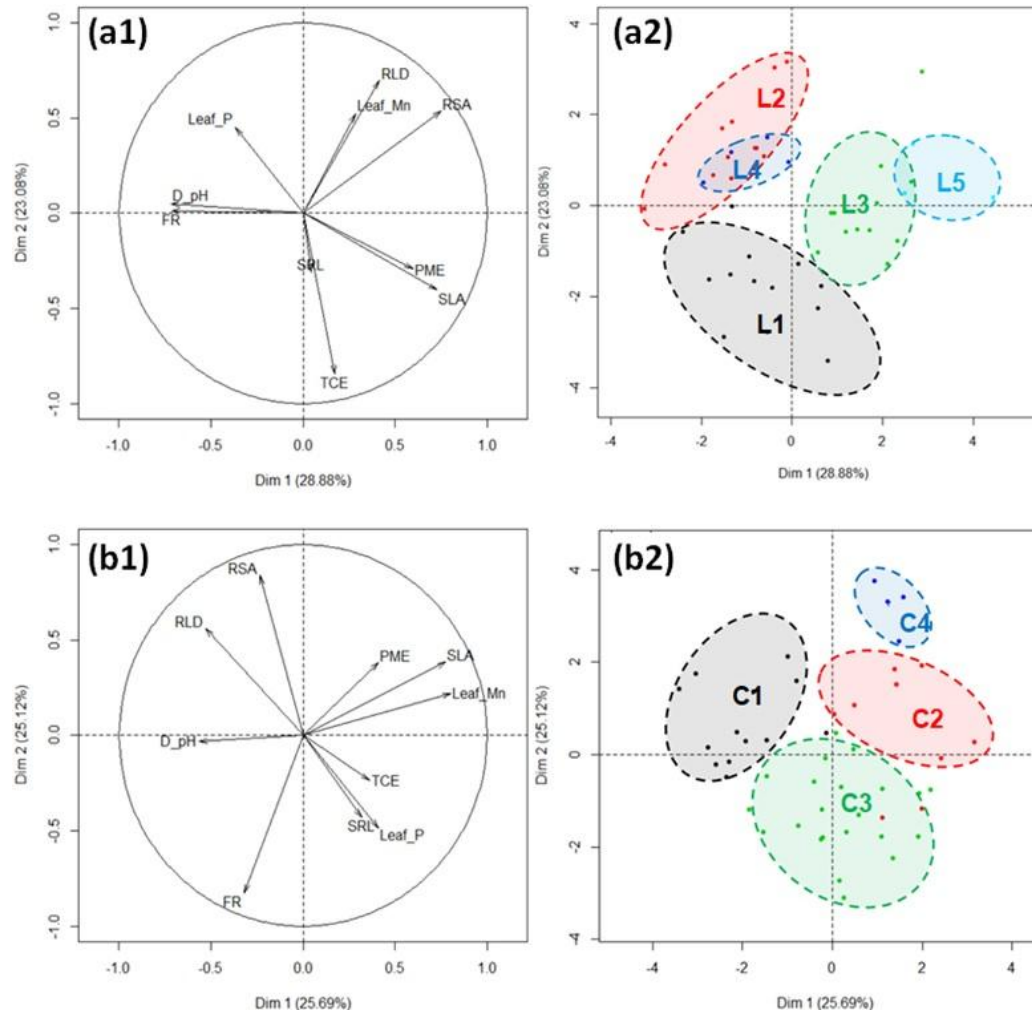
Soil type	Models	AICc	$\Delta$ AICc
Retisol	FR + Leaf [Mn] + PME	-18.8	0.0
	FR + Leaf [Mn]	-17.4	1.4
	FR	-16.5	2.3
	SLA	-14.5	4.3
	PME	-12.5	6.2
	Leaf [Mn]	-8.2	10.6
Calcaric Cambisol	FR + $\Delta$ pH + SLA + RSA	-43.4	0.0
	$\Delta$ pH + SLA + RSA	-39.5	3.9
	FR + SLA + RSA	-39.5	3.9
	SLA	-35.1	8.4
	RSA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

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

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

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

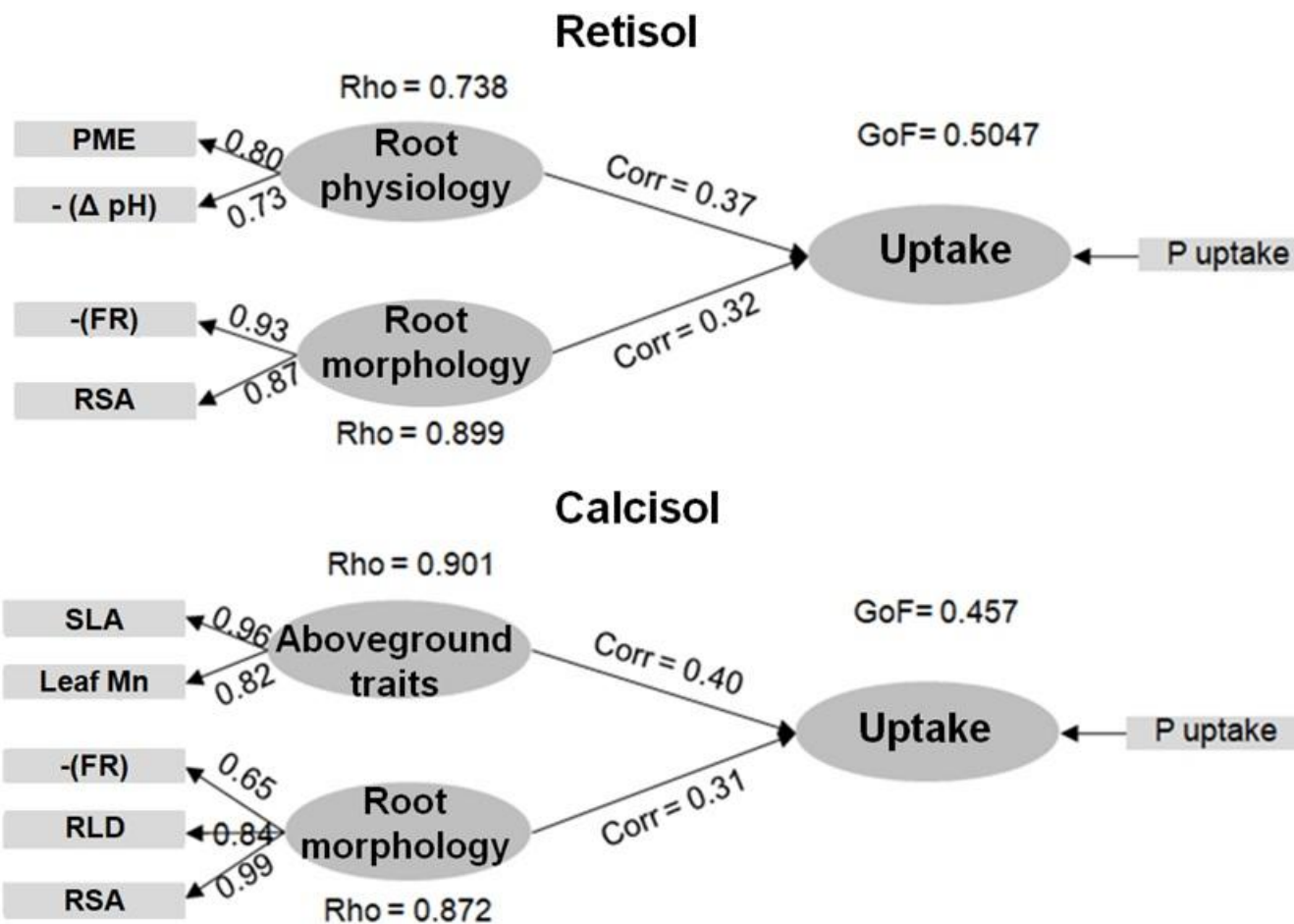
575 <sup>a</sup> Kruskal-Wallis test. Analysis performed on log transformed data. FR: Fine root percentage (<0.5 mm diameter), Leaf [Mn]: Leaf manganese concentration ( $\mu\text{g Mn g}^{-1}$ ), Leaf  
576 [P]: Leaf phosphorus concentration ( $\text{mg P g}^{-1}$ ), PME : Phosphomonoesterase activity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



579  
580

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

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



584  
585 **Fig.2** Partial least square- path models predicting phosphorus uptake  
586

587 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  
588 coefficient. Variables negatively transformed indicated by -(variable). GoF= Goodness of Fit of the model. ΔpH: change in rhizosphere pH; FR: percentage roots with  
589 diameters less than 0.5 mm; Leaf Mn: foliar manganese concentration; PME: acid phosphomonoesterase activity; RLD: root length density; RSA: root surface area; SLA:  
590 specific leaf area.