

Relationships between carboxylate-based nutrient-acquisition strategies, phosphorus-nutritional status and rare earth element accumulation in plants

Oliver Wiche, Christine Dittrich, Olivier Pourret, Nthati Monei, Juliane

Heim, Hans Lambers

▶ To cite this version:

Oliver Wiche, Christine Dittrich, Olivier Pourret, Nthati Monei, Juliane Heim, et al.. Relationships between carboxylate-based nutrient-acquisition strategies, phosphorus-nutritional status and rare earth element accumulation in plants. Plant and Soil, 2023, 10.1007/s11104-023-06049-9. hal-04087722

HAL Id: hal-04087722 https://hal.science/hal-04087722

Submitted on 3 May 2023

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 Relationships between carboxylate-based nutrient-acquisition strategies, phosphorus-nutritional

2 status and rare earth element accumulation in plants

- 3
- 4 Oliver Wiche^{ab}, Christine Dittrich^a, Olivier Pourret^b, Nthati Monei^{ad}, Juliane Heim^e, Hans Lambers^c
 5
- ⁶ ^aTechnische Universität Bergakademie Freiberg, Institute of Biosciences, Biology/Ecology Group,
- 7 Freiberg, Germany
- 8 ^bUniLaSalle, AGHYLE, Beauvais, France
- 9 ^cSchool of Biological Sciences and Institute of Agriculture, University of Western Australia,

10 Perth, Western Australia.

- 11 ^dTallinn University of Technology, Institute of Geology, Tallinn Estonia
- 12 ^eTechnische Universität Bergakademie Freiberg, Institute of Biosciences, Environmental Microbiology
 13 Group, Freiberg, Germany
- 14
- 15 Abstract

16 Background and Aims We explored how phosphorus (P) availability influences accumulation of rare 17 earth elements (REE) in plant species with different P-acquisition strategies beyond the commonly 18 explored REE-phosphate precipitation. Methods Two P-efficient carboxylate-releasing lupin species 19 (Lupinus albus, and L. cosentinii) and four species with less carboxylate release under P-deficiency 20 (Triticum aestivum, Brassica napus, Pisum sativum, Cicer arietinum), were cultivated with a split-root 21 system on two sand types. Phosphorus availability was controlled on one root side by watering the plants 22 with 100 µM P or 0 µM P solutions. Carboxylate release and changes in pH were measured on both 23 sides. Concentrations of nutrients, cadmium (Cd), aluminum (Al), light REE (LREE: La-Eu), and heavy 24 REE (HREE: Gd-Lu, including Y) in roots and shoots were analyzed by ICP-MS. Results P-deficient 25 T. aestivum, B. napus and C. arietinum did not respond with elevated carboxylate release. These species 26 accumulated more REE when the P supply was low and higher REE concentrations were proportional 27 to declining plant growth. However, P. sativum, L. albus and L. cosentinii accumulated less REE when 28 P supply was low. Plants that strongly acidified the rhizosphere and released low quantities of

29	dicarboxylates accumulated more REE (with higher LREE/HREE ratios) than species that released
30	tricarboxylates. Conclusion Our findings suggest that REE accumulation strongly depended on
31	rhizosphere acidification, in concert with the amount and composition of carboxylates determining the
32	exclusion of REE-carboxylate complexes. Leaf REE signatures may offer a promising ionomics
33	screening tool for carboxylate release into the rhizosphere.
34	
35	Keywords: element exclusion, rhizosphere chemistry, complexation, carboxylates
36	
37	
38	
39	
40	
41	
42	
43	
44	
45	
46	
47	
48	
49	
50	
51	
52	
53	
54	
55	
56	

57 Introduction

58

59 Root carboxylate release is an essential plant strategy to access sparingly-available soil nutrients, especially inorganic phosphate (Pi), iron (Fe), and manganese (Mn) (Shane and Lambers 2005; Lambers 60 2022). Plants have adapted to conditions of heterogeneously distributed and sparsely-available soil 61 62 resources and evolved strategies to influence the properties of the soil surrounding their roots (rhizosphere) to create an environment more conducive for nutrient acquisition (rhizosheath). In addition 63 64 to mutualistic interactions with bacteria and fungi, and alteration of root morphology (Honvault et al. 65 2021a, 2021b), the most profoundly studied traits involved in direct root-soil interactions include the 66 acidification of the rhizosheath and release of chelating carbon compounds such as carboxylates 67 (Lambers et al. 2015; Honvault et al. 2021b; Lambers 2022). Rhizosphere acidification in the presence 68 of carboxylates increases the solubility and availability of many essential or beneficial elements, 69 including P, Fe, Mn, Cu, Zn and Si, by dissolution, complexation and ligand-exchange reactions (de 70 Tombeur et al. 2021; Lambers 2022). The ability to mobilize Pi and micronutrients in the rhizosphere 71 varies considerably among plant species, functional plant groups (Neumann et al. 2000; Lambers et al. 72 2013; Lambers et al. 2015) or even genotypes of specific species (Krasilnikoff et al. 2003; Pang et al. 73 2018). Plant species adapted to P-impoverished or P-sorbing soils, of which Proteaceae and some grain 74 legumes such as Lupinus albus have been most profoundly studied, respond to P deficiency by increased 75 release of citrate and malate (Neumann and Römheld 2000; Pearse et al. 2006). In contrast, non-76 mycorrhizal phosphophilous species in the Brassicaceae, Chenopodiaceae, Urticaceae and some cereals 77 such as Triticum aestivum, do not respond to P deficiency with elevated carboxylate release (Pearse et 78 al. 2006; Lambers 2022).

Although carboxylate-based P-acquisition strategies are regulated by plant P status and predominantly target the acquisition of essential mineral nutrients, the resulting chemical changes in the rhizosphere are non-element-specific (Lambers et al. 2015). That means, while nutrient deficiency triggers a shift in metabolism towards elevated proton and carboxylate release, the compounds released solubilize not only nutrients, but also mobilize a number of non-essential elements in the rhizosphere, impacting their chemical speciation and availability to plants as demonstrated for Cd, Pb, Ge and rare earth elements

85 (REEs) (Wenzel 2009, Wiche et. 2016a, 2016b). In this respect, REEs are particularly interesting to 86 study, because they i) are present in almost all soils at concentrations similar to essential plant nutrients 87 (Reimann et al. 2003; Wiche et al. 2017a), ii) share chemical similarities with essential nutrients, mainly 88 Ca (Tyler 2004; Brioschi et al. 2013), and iii) strongly interact with nutrient-bearing soil minerals 89 (phosphates, Fe-oxyhydroxides), but are neither essential to plants nor strongly toxic (Tyler 2004; 90 Davranche et al. 2017). The REEs comprise a group of 16 elements from the lanthanide series, including 91 lanthanum, yttrium (Y) and scandium (Sc) that are widespread in the earth's crust with concentrations 92 that vary from 66 μ g g⁻¹ (Ce), 30 μ g g⁻¹ (La) and 28 μ g g⁻¹ (Nd) to 0.3 μ g g⁻¹ (Lu) (McLennan 2001; Davranche et al. 2017). As a unique feature in this group, all 16 REEs exhibit ionic radii similar to Ca^{2+} ; 93 94 however, under most pedologically-relevant conditions, REEs form trivalent cations (Wyttenbach et al. 95 1998), which strongly interact with phosphate and other negatively charged soil constituents (Diatloff 96 et al. 1993; Cao et al. 2001; Li et al., 2014). In particular, they can form stable complexes with dissolved 97 organic compounds (Pourret et al. 2007; Wiche et al. 2017b), and their stability depends on the nature 98 of the ligand and the REE involved. There are slight differences in ionic radii from light REEs (LREE: 99 La to Eu) to heavy REEs (HREE: Gd to Lu, including Y), leading to differences in their sorption and 100 complexation behavior in soil and their availability in the rhizosphere (Khan et al. 2016; Schwabe et al. 101 2021; Monei et al. 2022). For REEs in the soil solution, it is generally assumed that uptake of REE³⁺-102 ions is mediated mainly by Ca^{2+} , Na⁺- and K⁺-channels (Han et al. 2005; Brioschi et al. 2013), while 103 REE-carboxylate complexes are excluded, relative to free ionic forms (Han et al. 2005; Wiche et al. 104 2017b). After root sorption, due to the element's higher reactivity, the biogeochemical behavior of REEs 105 in the soil-plant system is not simply analogous to Ca^{2+} , but may resemble that of other trivalent metals, 106 particularly Al³⁺ (Ma and Hiradate 2000). Thus root-shoot transport of REEs depends on their mobility 107 within the plant (Kovarikova et al. 2019), most likely governed by cell-wall absorption, phosphate 108 deposition and intracellular complexation with carboxylates (Ma and Hiradate 2000). Based on the 109 above, plant species that deploy a carboxylate-based nutrient-acquisition strategy will likely exhibit 110 differences in REE sorption. The P status of the plants and the quantity and composition of the 111 compounds released should influence the processes during the mobilization of non-essential elements 112 and their uptake.

113 In the present study, we conducted a split-root experiment with two P-efficient carboxylate-releasing 114 lupin species (Lupinus albus and Lupinus cosentinii) that typically show carboxylate release under low 115 P supply, and four species (Triticum aestivum, Brassica napus, Pisum sativum, Cicer arietinum) that 116 lack the ability to respond to P deficiency with elevated carboxylate release (Pearse et al. 2006). A split-117 root approach was used to exclude the direct effects of P addition on REE availability, i.e. by 118 precipitation as REE-phosphates (Fehlauer et al. 2022; Liu et al. 2022). Thus, one root half received all 119 essential plant nutrients except phosphate, which was supplied to the other root half only. Root 120 carboxylate release and shoot element concentrations (selected nutrients, aluminum, cadmium, REE) 121 were measured, to explore the relation between P nutrition and accumulation of non-essential elements, 122 including total REE uptake and LREE/HREE ratios. If we would be able to show such a correlation, this 123 would offer the possibility to use shoot REE signatures to proxy the involvement of carboxylates in 124 nutrient acquisition.

- 125
- 126 Methods
- 127
- 128 Substrates for plant cultivation
- 129

130 In this experiment, 120 pots (7 x 7 x 18 cm) were filled with 1.2 kg of sand. Half of the pots (60 pots) were filled with quartz sand (0.1–0.4 mm grain size, 1500 kg m⁻³), while the other half was filled with a 131 132 mixture of 75% of quartz sand and 0.25% of river sand (0.4–2 mm grain size, 1,320 kg m⁻³). Here, a 133 second sand type was added to increase the amount of potentially plant-available elements to one half 134 of the split-root systems. The quartz sand had a pH of 5.6 (water/solid 1/10) and 1.1 ± 0.5 mg kg⁻¹ 135 calcium lactate-extractable P (van Laak et al. 2018), whereas the mixed sand had a pH of 5.9 and $2.1 \pm$ 0.3 mg kg⁻¹ P. In both sand types, the total element concentrations were similar (Table 1); however, the 136 137 sand types differed regarding the distribution of elements in potentially plant-available element fractions 138 indicated by a sequential extraction analysis considering the distribution of elements in five 139 operationally-defined soil fractions according to Wiche et al. (2017a) (Table 1). In these fractions, the 140 mixed sand was characterized by higher concentrations of P, Mn and Fe (Table 1). Furthermore, the

141 quartz sand showed higher concentrations of mobile/exchangeable and acid-soluble Al and higher 142 concentrations of mobile/exchangeable LREE. Thus, in quartz sand, these elements are more easily 143 accessible by roots than in mixed sand. However, Al and both LREE and HREE were generally more 144 concentrated in the mixed sand, especially in the more stable fractions 4 and 5, which were also the 145 significant element-bearing fractions of Cd (Table 1). The LREE / HREE ratios in both sand types were 146 > 1 (Table 1). In particular, quartz sand exhibited a 12% higher LREE / HREE ratio in Fractions 1 147 (mobile/exchangeable) and a 15% and 33% higher LREE/HREE ratio in Fractions 4 and 5, respectively, 148 where the elements are predominantly bound to amorphous and crystalline structures of oxides and 149 oxide-hydroxides (Table 1). In Fractions 2 and 3, however, the LREE/HREE ratios were similar between 150 the two substrates.

- **Table 1** Total element concentrations and distribution of elements in exchangeable (F1), acid-soluble (F2), oxidizable (F3) and moderately-reducible (F4) fractions
- $(\mu g g^{-1})$ according to Wiche et al. (2017) determined by a sequential extraction method (mean \pm sd; n = 10). Differences in means between the two sand types are

Substrate	Fraction	Р	Mn	Fe	Al	Cd	LREE	HREE
Quartz sand	Total	$358 \pm 50a$	466 ± 245	1773 ± 635	5279 ± 1134	1.44 ± 0.56	12.3 ± 3.4	3.6 ± 0.9
	1	< 0.5	$0.4\pm0.1b$	0.8 ± 0.2	$2.1\pm0.6a$	< 0.05	$0.40\pm0.03a$	0.10 ± 0.01
	2	< 0.5	$0.14\pm0.12b$	$6.5 \pm 1.3b$	$18\pm 2a$	< 0.05	$0.15\pm0.03b$	$0.05\pm0.01b$
	3	123 ±10b	$0.19\pm0.10b$	$7.9\pm2.0b$	$4.9\pm0.6b$	< 0.05	$0.10\pm0.04b$	$0.21\pm0.09b$
	4	26.2 ± 2.9	$0.78 \pm 0.09 \mathrm{b}$	89 ± 14	$37.8\pm2.6a$	$0.09\pm0.01b$	$0.60\pm0.06b$	$0.17\pm0.03b$
	5	<0.5b	$0.64 \pm 0.14b$	$71 \pm 31b$	$73.7\pm7.2b$	$0.09\pm0.01b$	$0.81\pm0.13b$	$0.14\pm0.03b$
Mixed sand	Total	$281\pm36b$	334 ± 125	2174 ± 748	5617 ± 1990	1.38 ± 0.34	13.8 ± 1.9	5.0 ± 1.1
	1	< 0.5	$2.2\pm0.5a$	1.0 ± 0.4	$1.4\pm0.2b$	< 0.05	$0.33\pm0.04b$	0.09 ± 0.01
	2	< 0.5	$0.71 \pm 0.42a$	$10.3 \pm 2.4a$	$13\pm3b$	< 0.05	$0.19\pm0.03a$	$0.06\pm0.01a$
	3	$165 \pm 16a$	$1.87 \pm 1.12a$	$12.3\pm2.7a$	$7.6 \pm 1.1a$	< 0.05	$0.18\pm0.05a$	$0.41 \pm 0.17a$
	4	23.8 ± 2.6	$2.17\pm0.86a$	95 ± 19	$32.9\pm3.1b$	$0.11\pm0.01a$	$0.76\pm0.08a$	$0.25\pm0.04a$
	5	$8.0 \pm 1.7a$	4.44 ± 1.69a	$518 \pm 149a$	$98.7 \pm 14.2 \mathrm{a}$	$0.12 \pm 0.01a$	$1.03 \pm 0.19a$	$0.24\pm0.06a$

153 identified by t-tests with Bonferroni correction. Means with different letters are significantly different at $\alpha = 5\%$



157 Seeds of Triticum aestivum cv Arabella, Brassica napus cv Genie, Pisum sativum cv Karina, Cicer 158 arietinum cv Kabuli, Lupinus albus cv Feodora, and Lupinus cosentinii cv were surface sterilized by 159 washing the seeds with 0.5% sodium hypochlorite (NaOCl) for 3 min, followed by rinsing with 160 deionized water. Seeds were germinated in Petri dishes in a climate chamber at 20°C. After germination 161 and development of seminal roots, the seedlings were transferred to a hydroponic culture with a 1/20 162 strength Hoagland solution (Arnon and Stout 1939), 22°C room temperature, relative humidity 60% and 600 μ mol m⁻² s⁻² photosynthetically active radiation. After one week, the primary roots of *B. napus*, *P.* 163 164 sativum, C. arietinum, L. albus, and L. cosentinii were cut 1 cm below the first lateral roots to obtain a 165 split root system by stimulation of root branching and lateral root development (Saiz-Fernandez 2021). 166 Triticum aestivum developed several seminal roots; thus, the abovementioned procedure was 167 unnecessary, and the roots could easily be diverted into different compartments. After cutting, all plants 168 were transferred back into the hydroponic solution and cultivated for another 10 days to allow the plants 169 to recover (Saiz-Fernandez 2021).

170 Plant individuals with similarly developed root systems were transferred from hydroponic culture into 171 the previously prepared pots filled with sand. Each experimental unit consisted of one plant with a split 172 root system where one part of the root system was placed in a pot with quartz sand and the other part 173 into a pot with mixed sand. The pots were connected with clamps, and the seedlings were stabilized with 174 a stick to support the shoot growing between the two pots. In total, from each plant species, 10 175 experimental units were prepared. The plants were grown in a growth chamber at 22°C and 65% 176 humidity, 600 μ mol m⁻² s⁻¹ photosynthetically active radiation and watered with a 1/20 strength 177 Hoagland solution containing all essential mineral nutrients, except P. After one week of growth and 178 allowing the plants to extend their roots deeper in the sand substrates, the experimental units were 179 watered with two different nutrient solutions containing either all essential plant nutrients according to 180 a 1/10 strength Hoagland solution except P (P0), or all mineral elements contained in the previous 181 solution with the addition of 100 μ M P (P+). Half of the experimental units were watered with P0 182 solutions at both root sides (50 mL in each pot), whereas the other half received P+ solutions at the root 183 side growing in quartz sand (50 mL) and P0 solutions at the root side growing in mixed sand (50 mL).

184 The addition of treatment solutions was continued every second day over a period of five weeks. Each 185 P treatment was replicated fivefold for each plant species, and the different species and treatments were 186 spatially distributed in a fully randomized design.

187

- 188 Rhizosphere properties and exudate collection
- 189

190 After five weeks, the plants were removed from the sand and carefully shaken to remove loose sand 191 particles. Sand adhering to the root surface was collected by washing the roots with 20 mL of deionized 192 water until 1 g of rhizosheath was obtained. The sand was left in the washing solution for 1 h until the 193 pH was measured using a pH electrode. If necessary, the root was washed a second time without 194 collecting the solution or sand material to remove the remaining sand entirely. The plants were 195 transferred with their individual root systems into a 200 mL sterile Erlenmeyer flasks filled with 100 196 mL of a 2.5 µM CaCl₂ solution. This allowed the collection of root exudates depending on plant species 197 and P-treatment for each root system separately. The plants in the collection solutions were placed back 198 into the growth chamber and allowed to release root exudates over a time period of 3 h. Immediately 199 after the collection, the resulting solutions were analyzed using ion chromatography. After that, the 200 plants were separated into roots and shoots. Shoots were washed for 1 min with deionized water. The 201 split roots were separately washed for 5 min with ice-cold CaCl₂ solution (5 mM) and 1 min with 202 deionized water to remove adsorbed ions from charged root cell structures (Han et al. 2005). Finally, the 203 shoots and roots were dried at 60°C for 48 h, weighed and stored in centrifuge tubes until being analyzed 204 by inductively coupled plasma mass spectrometry (ICP-MS).

205

206 Determination of carboxylates and element concentrations

207

The dried plant material was ground to a fine powder using a centrifugal mill equipped with a titanium rotor (Retsch ZM 100) and stored in centrifuge tubes. Afterwards, microwave digestion (Ethos plus 2, MLS, Leutkirch, Germany) was carried out with 0.1 g of subsample taken from the ground biomass and measured in duplicate. Samples were mixed with 1.6 mL nitric acid (65% suprapure) and 0.6 mL hydrofluoric acid (4.9% suprapure) and heated to 220°C in a microwave, according to Krachler et al. 213 (2002). Concentrations of P, Fe, Mn and REEs (Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb 214 and Lu) from the diluted digestion solutions and soil solutions were determined by ICP-MS (XSeries 2, Thermo Scientific, Dreieich, Germany) using 10 µg L⁻¹ rhodium and rhenium as internal standards. 215 216 Possible interferences were monitored and corrected if necessary (Pourret et al. 2022). 217 Concentrations of acetate, fumarate, glutarate, malate and citrate in the collection solutions were 218 determined by ion chromatography equipped with conductivity detection (ICS-5000, 4 mm system, 219 Thermo Scientific, Dreieich, Germany). Inorganic and organic acid anions were separated at 30°C on 220 an IonPac® AS11-HC column (Thermo Scientific, Dreieich, Germany) using gradient elution with

- sodium hydroxide as eluent and a flow rate of 1.0 mL min⁻¹.
- 222
- 223 Data processing and statistical analysis
- 224

225 Concentrations of LREEs and HREEs in the plant and soil samples were calculated as sums of La, Ce, 226 Pr, Nd, Pm, Sm, Eu (LREEs) and Gd, Tb, Y, Ho, Er, Yb, Tm, Lu (HREEs) according to Tyler (2004). 227 Significant differences among means of element concentrations in soil fractions, carboxylate 228 concentrations of P+ and P0 plants, and element concentrations in plant parts cultivated with different P 229 supply were compared by t-test with Bonferroni adjustment of p-values using IBM SPSS Statistics 25. 230 Carboxylate release and element concentrations in different root parts of the same plants were compared 231 by a t-test for non-independent samples at $\alpha = 5\%$. Element concentrations, contents and root carboxylate 232 release among plant species within a certain P-treatment were compared by one-way analysis of variance 233 (ANOVA) followed by Tukey's HSD post-hoc test. Prior to the analysis, the data were checked for 234 homogeneity of variances using Levene's-test. In case the assumption of homogeneity was violated, the 235 data were log-transformed. If the assumption was still violated, significant differences between means 236 were identified using Welch's ANOVA at $\alpha = 5\%$.

- 238 Results
- 239
- 240 Plant growth and biomass responding to P-supply
- 241

The dry biomass varied considerably among the tested plant species (Figure 1). *Brassica napus* accumulated the most biomass (3.1 g) when P was supplied, and *T. aestivum* accumulated the least biomass when no P was supplied (0.2 g) (Fig. 1A). Phosphorus supply increased the total dry mass (shoot and root mass) of *T. aestivum*, *B. napus*, *C. arietinum* and *L. albus* by 65%, 52%, 27%, and 56%, respectively. In contrast, the total biomass of *P. sativum* and *L. cosentinii* did not respond to P supply (Fig. 1A).

248 Considering the shoot biomass of plants, the addition of P did not significantly ($\alpha = 5\%$) affect the shoot 249 mass of P. sativum, C. arietinum, and L. cosentinii, and there were no differences among P. sativum, L. 250 cosentinii and C. arietinum (Fig. 1B). However, L. albus, B. napus and T. aestivum strongly responded 251 (p < 0.01) to elevated P-supply with 39%, 52%, and 88% greater shoot biomass (Fig. 1B). Considering 252 the whole root system, including both root parts growing in quartz sand and mixed sand, the addition of 253 P did not significantly affect root biomass within a plant species, but tended to increase (p = 0.14) total 254 root mass in L. cosentinii, by 124% compared with P-deficient plants (Fig. 1C). There were no 255 differences between plant species when the plants were externally supplied with P (Fig. 1C). However, 256 in P0-treated plants, L. albus and C. arietinum showed the greatest root mass, and the lowest root mass 257 was found for L. cosentinii (Fig. 1C).

258 Considering the development of the split root systems in different sand types, all species tended to have 259 a greater root mass in mixed sand, especially T. aestivum and L. albus, which showed 200% and 60% 260 more root mass when no P was supplied and C. arietinum (125% more biomass in mixed sand) when P 261 was provided. Without the addition of P, the root mass ratio varied significantly among the species 262 showing decreasing ratios from T. aestivum > C. arietinum > P. sativum, L. albus, L. cosentinii > B. 263 napus (Fig. 1D). Addition of P significantly reduced the root/shoot ratio in T. aestivum, B. napus and L. 264 albus by 45%, 25% and 17%, respectively, while in the other plant species, there were no effects (C. 265 arietinum) or slightly increasing trends (P. sativum, L. cosentinii). When the plants received solutions 266 containing 100 µM P, the root mass ratio was similar in T. aestivum, P. sativum, C. arietinum and L. 267 cosentinii, but lowest in B. napus (Fig. 1D).



269 Fig. 1 Shoot mass and root mass (g) of the two root halves growing in quartz sand (Q) and mixed sand 270 (M) and root mass ratio considering both root halves depending on treatment with 100 µM P (P+) and 271 no P (0P). The bars represent means \pm se (n = 5). ANOVA identified differences among species (capital 272 letters) following Tukey's HSD post-hoc test at $\alpha = 5\%$. Significant differences in shoot mass and root 273 mass ratios between different P-treatments are indicated by asterisks (**p < 0.01; *p < 0.05; (*)p274 <0.1).Differences in root masses between the root halves within a P treatment are indicated with 275 lowercase letters. Capital letters denote differences among the species within a P-treatment. Means with 276 the same letters are not significantly different at $\alpha = 5\%$

Shoot [P] of plants watered with 100 μ M P ranged from 1.21 mg g⁻¹ (B. napus) to 2.46 mg g⁻¹ (T. 280 281 aestivum) (Table 2). Triticum aestivum and L. cosentinii showed substantially higher [P] in shoots than 282 all other investigated species did. Shoot [P] of T. aestivum, B. napus, P. sativum, L. albus and L. 283 cosentinii responded to a reduction in P supply by a 57%, 19%, 13%, 12% and 20% decrease of shoot 284 [P], respectively, compared with plants treated with high P (100 µM P). Shoot [P] in C. arietinum was 285 almost unchanged; however, under conditions of low P supply, C. arietinum, L. albus and L. cosentinii 286 still displayed the highest shoot [P] compared with *B. napus* and *T. aestivum*. 287 Concerning the measured micronutrients, L. albus exhibited the highest [Mn] and [Fe], irrespective of

P-supply. The addition of low P doses tended to decrease shoot [Mn] in *T. aestivum*, *B. napus*, and *P. sativum* and significantly decreased [Fe] in *P. sativum*. In contrast, shoot [Mn] in low-P plants of *L. albus* and *L. cosentinii* were consistently higher than those in plants that received P with the watering solution (Table 2).

292

Table 2 Concentration of nutrients in shoots of six plant species cultivated under split-root conditions

294 (means \pm sd; n = 5) and addition of 100 μ M P (P+) and no P in the treatment solution (0P). Capital letters

denote significant differences among the species within a P tre	atment
---	--------

Species	P-treatment	Р	Mn	Fe
		mg g ⁻¹	μg g ⁻¹	
Triticum aestivum	P0	$1.06 \pm 0.05B$	$160 \pm 35B$	$122 \pm 42A$
	P+	$2.46 \pm 1.03 A$	$206\pm29B$	$99 \pm 22 AB$
	p-value	0.02	0.06	0.32
Brassica napus	PO	$0.98\pm0.06B$	97 ± 17C	$39 \pm 3B$
_	P+	$1.21 \pm 0.12B$	$114 \pm 13BC$	$45 \pm 8B$
	p-value	0.05	0.22	0.14
Pisum sativum	PO	$1.14\pm0.09AB$	$88 \pm 18BC$	$67 \pm 10BC$
	P+	$1.31\pm0.02B$	$100 \pm 36BC$	$97 \pm 17 AB$
	p-value	0.11	0.50	< 0.01
Cicer arietinum	PO	$1.23 \pm 0.17 A$	$63 \pm 18C$	84 ± 31BC
	P+	$1.30\pm0.11B$	$53 \pm 21C$	97 ± 43 AB
	p-value	0.56	0.42	0.59
Lupinus albus	PO	$1.25 \pm 0.15 A$	$452\pm96A$	$160 \pm 71A$
	P+	$1.42\pm0.071B$	$413 \pm 51A$	$107 \pm 30A$
	p-value	0.05	0.25	0.15
Lupinus cosentinii	PO	$1.39 \pm 0.17 A$	121 ± 58BC	88 ± 18BC
	P+	$1.73 \pm 0.16 A$	$96 \pm 38BC$	$87 \pm 20 AB$
	p-value	0.03	0.56	0.91
Species	P0	< 0.001	< 0.001	< 0.01
-	P+	< 0.01	< 0.001	< 0.01

296

300 Root [P], [Mn] and [Fe] varied substantially among species, P supply and the root part considered (roots 301 growing in quartz sand and mixed sand, respectively) (Table 3). Considering the root part growing in 302 quartz sand, where P-supply was controlled, the addition of low-P solutions (OP) decreased root [P] of 303 all species by 10–25% compared with plants treated with 100 µM P (P+). This effect was strongest in 304 T. aestivum, B. napus, P. sativum and C. arietinum (p < 0.05) and somewhat weaker in L. albus and L. 305 cosentinii (p > 0.05). When P supply was high, T. aestivum, B. napus, C. arietinum and L. cosentinii 306 showed higher root [Mn] than L. albus, and the reduction of P supply did not influence [Mn] of roots in 307 quartz sand. However, P-deficient *L. cosentinii* had a 65% higher [Mn] than P-supplied plants (p = 0.12). 308 Triticum aestivum exhibited the highest [Fe] of all species, irrespective of P treatment, and B. napus the 309 lowest. Reduction of P supply increased root [Fe] in B. napus and L. albus by 161% and 44%, 310 respectively.

311 In the corresponding mixed sand root part of P-supplied plants, root [P] was the highest in B. napus and 312 P. sativum and the lowest in T. aestivum. When P supply was reduced at the root side in quartz sand, 313 root [P] also declined significantly in the mixed sand root part of T. aestivum (19%) and L. albus (18%), 314 but it was unchanged in the other species. Considering both P treatments, roots in mixed sand of T. 315 aestivum and C. arietinum showed consistently lower [P] than roots growing in quartz sand, while in B. 316 napus and P. sativum root [P] only differed when P was added to the root half in quartz sand (higher [P] 317 in quartz sand than in mixed sand). Lupinus albus and L. cosentinii did not show any differences in root 318 [P] between the roots when P was added, nor in situations of P deficiency. Concerning the 319 micronutrients, the root half in mixed sand generally responded more strongly to the P-treatment than 320 the root half growing in quartz sand (Table 3). Specifically, the reduction in P supply increased [Mn] in 321 B. napus (45%), P. sativum (83%), C. arietinum (54%), L. albus (20%) and L. cosentinii (81%).

- 323
- 324
- 325
- 326

- 327 **Table 3** Nutrient concentrations in roots of six species cultivated under split-root conditions on two sand
- 328 types, quartz sand and mixed sand (means \pm sd; n = 5). The plants received 100 μ M P (P+) or no P (0P)
- 329 in quartz sand. Capital letters denote differences among plant species within a P treatment, and lowercase
- 330 letters denote differences between the root halves for a specific element

		Root part with l	P supply (quar	tz sand)	Root part without P supply (mixed sand		
Species	treatmen	tP	Mn	Fe	Р	Mn	Fe
		mg g ⁻¹	μg g ⁻¹		mg g ⁻¹	μg g ⁻¹	
T. aestivum	P0	$1.34 \pm 0.25 aAB$	$103 \pm 28 \text{bA}$	8345 ± 2889aA	$0.93 \pm 0.13 bC$	$175 \pm 49 aA$	$1784 \pm 440 bA$
	P100	$1.78 \pm 0.23 aA$	$131 \pm 27 \text{bAB}$	$6270 \pm 2032 aA$	$1.15 \pm 0.09 bC$	$252\pm45aA$	$2614\pm585 bA$
	p-value	0.02	0.14	0.13	0.01	0.06	0.04
B. napus	P0	$1.54 \pm 0.19 A$	$148 \pm 83A$	$1243 \pm 697B$	$1.53\pm0.10A$	$97 \pm 23B$	$391 \pm 97C$
_	P100	1.79 ± 0.11 aA	$86 \pm 40 AB$	$476 \pm 132C$	$1.56\pm0.06 bA$	$67 \pm 21C$	$316 \pm 48D$
	p-value	0.03	0.23	0.04	0.65	0.10	0.31
P. sativum	P0	$1.52\pm0.13A$	152 ± 109 AB	$1692 \pm 792B$	$1.39\pm0.08A$	$165 \pm 71 \text{AB}$	$1213 \pm 434 \text{AB}$
	P100	1.68 ± 0.04 aA	$64 \pm 35B$	$1489 \pm 34aB$	$1.35\pm0.07bB$	90 ± 26BC	$1100 \pm 89 bB$
	p-value	0.06	0.24	0.68	0.44	0.08	0.63
C. arietinun	ı P0	$1.32 \pm 0.09 aAB$	$129 \pm 43 \text{bA}$	$1515 \pm 260 \mathrm{aB}$	$1.15\pm0.06bB$	$205\pm 30 aA$	$1014\pm82 bB$
	P100	$1.85 \pm 0.30 aA$	$213\pm126A$	$1909 \pm 799 aB$	$1.21 \pm 0.07 bBC$	$133 \pm 17B$	$758 \pm 171 bC$
	p-value	< 0.01	0.20	0.38	0.15	< 0.01	0.02
L. albus	P0	$1.10\pm0.26B$	$47\pm8B$	$1325 \pm 250 \mathrm{aB}$	$1.08\pm0.04B$	$43\pm8C$	$632 \pm 79 bB$
	P100	$1.35\pm0.08B$	$44\pm14B$	$919 \pm 145 aB$	$1.31\pm0.17BC$	$36 \pm 5C$	$790 \pm 109 bC$
	p-value	0.09	0.72	0.01	0.03	0.18	0.25
L. cosentinii	P0	$1.38\pm0.17A$	$134 \pm 23A$	$2691 \pm 2035B$	$1.20\pm0.04B$	$123 \pm 77 \text{AB}$	$1075 \pm 537 \text{AB}$
	P100	$1.63\pm0.45AB$	$81 \pm 53 AB$	$2610 \pm 1567B$	$1.30\pm0.05BC$	$68 \pm 21C$	$1084 \pm 344BC$
	p-value	0.31	0.12	0.95	0.10	0.10	0.97
Species	P0	0.04	< 0.01	0.02	< 0.001	< 0.001	< 0.001
	P100	0.05	0.01	< 0.001	< 0.001	< 0.001	< 0.001

332 Carboxylate release in response to P supply

333

334 Considering the quantity of carboxylates released by both root parts per root half and unit of time, B. 335 napus released by far the greatest amounts, irrespective of P treatment (Fig. 2A). In B. napus, T. aestivum 336 and P. sativum, the major portion (more than 98%) of the carboxylates released consisted of malate, and 337 citrate was only occasionally detected. In contrast, C. arietinum, L. albus and L. cosentinii released both 338 malate and citrate (Fig. 2A). Carboxylate release was not affected by P supply in T. aestivum and C. 339 arietinum. Brassica napus and P. sativum responded to a reduction in P supply with a decrease in 340 carboxylate release by 20% (p = 0.04) and 44% (p = 0.08), respectively. In contrast, in *L. albus* and *L.* 341 cosentinii, the reduction of P supply significantly increased total carboxylate release by 159% (p<0.01) 342 and 115% (p = 0.03), respectively, showing an increase of both malate and citrate, but especially of 343 citrate (Fig. 2A). Roots growing in mixed sand released greater amounts of carboxylates per unit time 344 in all tested species, except *B. napus*, which tended to release greater amounts of malate in quartz sand,

345 but only when this root part was supplied with P (Fig. 2B). Also, in the other species, there were 346 significant differences in the response of the different root halves to P supply. Triticum aestivum showed 347 no response in any of the root halves (Fig. 2B). Brassica napus, P. sativum and C. arietinum 348 predominantly responded in the root half in quartz sand, where P was added with the watering solution 349 and showed a significant reduction in carboxylate release (24%, 65% and 75%) at low P supply. In 350 comparison, in the root half in mixed sand, carboxylate release in P. sativum and C. arietinum was 351 unchanged or increased in B. napus by 80% when P supply in quartz sand was low. Also, L. albus and 352 L. cosentinii did not respond in the root half supplied with P but showed an increase of carboxylate 353 release from the root half in mixed sand only. Here, the exudation of malate increased by 121% and 354 320%, respectively, and citrate release increased by 192% and 870%, respectively, when P-supply was 355 low (Fig. 2B).

Carboxylate release per unit root mass showed far less variation depending on growth substrates and P supply (Fig. 2C). Mixed sand roots still tended to release more carboxylates per unit root mass. However, this trend was only observed in P-supplied *T. aestivum*, *C. arietinum* (irrespective of P-supply) and *L. albus*, but in the latter species only when P was lacking (Fig. 2C). Additionally, in both *C. arietinum* and *L. albus*, exudation rates were affected by P supply, showing less carboxylate release from the root half in quartz sand (*C. arietinum*: 90% decrease) or increasing exudation from the root half in mixed sand (*L. albus*: 105% increase) (Fig. 2C).

363

364 Rhizosheath acidification in response to P supply

365

366 In the rhizosheath of all plant species and treatments, the pH was significantly higher than that of the 367 unplanted control soil (Fig. 2D). The pH in the mixed sand rhizosphere was consistently higher (on 368 average 0.3 units considering all species) than that in quartz sand as a consequence of the initial pH of 369 the substrates used (Table 1); however, the pH of the substrates was altered depending on plant species, 370 root half and P supply (Fig. 2D). Considering data from both root halves, the rhizosphere pH of P-371 supplied plants of B. napus, L. albus and L. cosentinii (pH 7.1 \pm 0.2) was on average 0.5 units higher 372 than that of C. arietinum, P. sativum and T. aestivum (pH 6.6 ± 0.1). When the P supply was low, the 373 pH in the rhizosheath of B. napus was still highest and lowest in the soil of L. albus and L. cosentinii,

- 374 which was predominantly driven by a strong acidification at the root half in mixed sand. In contrast, in
- 375 *P. sativum* the pH was low in both root halves, irrespective of the P treatment.

Considering the different root halves under P+ conditions, the pH in the quartz sand rhizosheath was highest for *B. napus* and showed the pattern *B. napus* > *L. albus* = *L. cosentinii* > *P. sativum* = *T. aestivum*

378 = *C. arietinum*. When P was lacking, the rhizosphere pH of *B. napus*, *C. arietinum* and *T. aestivum* was

379 0.3 units lower (p < 0.05) but unchanged (around 6.7 \pm 0.2) for L. albus, L. cosentinii and P. sativum.

- 380 At the root half with mixed sand, the pH of *B. napus, L. albus* and *L. cosentinii* was much (7.2 ± 0.1)
- higher than that of *T. aestivum*, *C. arietinum* and *P. sativum* (6.8 ± 0.1) ($\alpha = 1\%$). Here, the low P supply
- reduced the pH in the rhizosheath of *L. albus* and *B. napus* by 0.2 units and strongly reduced the pH in
- 383 the rhizosheath of *L. cosentinii*, by 0.6 units.



384

Fig. 2 Total carboxylate release per plant (A), (B) carboxylate release from root halves growing in quartz sand (Q) and mixed sand (M), (C) exudation rates from the different root halves, and (D) rhizosphere pH depending on treatment of plants with 100 μ M P (P+) or no P (0P) from the root half growing in quartz sand (means ± se, n = 5). Capital letters indicate significant differences between species within a P treatment. Small letters indicate i) differences among species concerning a specific carboxylate type within a P-treatment (A), ii) differences in carboxylate release between different root halves within a P-

391 treatment and species (B, C) or iii) differences in the pH between the same root half at different P supply 392 rates (D). Means with different letters are significantly different at $\alpha = 5\%$ identified by Tukey's HSD 393 post-hoc test

394

- 395 Shoot accumulation of non-essential elements
- 396

Shoot [Cd] was highest in *B. napus* and lowest in *C. arietinum* (Fig. 3). In contrast, [Al] was the lowest
in *B. napus*, and there were no differences among *T. aestivum*, *P. sativum*, *C. arietinum*, *L. albus*, and *L. cosentinii*. Regarding the REE concentrations, *P. sativum* had higher shoot [LREE] and [HREE] than
all other species did. HREE concentrations were similar in *T. aestivum*, *B. napus*, *C. arietinum*, *L. albus*,
and *L. cosentinii*; however, *B. napus*, *C. arietinum*, and *L. albus* showed higher [LREE] than *T. aestivum*and *L. cosentinii* (Fig. 3).

403 Phosphorus supply did not significantly affect shoot [A1] and [Cd] in the investigated species, except in 404 L. albus, which showed a 61% lower [Cd] when P supply was low. Concomitantly, L. albus and P. 405 sativum responded with a 42% and 49% decrease of [LREE] and a 42% and 44% decrease of [HREE] 406 at a low P supply. In contrast, T. aestivum and B. napus exhibited the highest [LREE] in P-deficient 407 plants, and [LREE] was 39% and 19% higher when P supply was high. At the same time, [HREE] were 408 unaffected by P supply in these two species. These changes in [LREE] and [HREE] altered the 409 LREE/HREE ratios of *B. napus* and *P. sativum*, which consistently exhibited higher LREE/HREE ratios 410 in P-deficient plants. In other species, no effects of P addition on the LREE/HREE ratios were observed, 411 except in L. cosentinii, which showed the opposite trend with a lower LREE/HREE ratio at a low P 412 supply (Fig. 3).

413 Considering the shoot element contents (calculated as shoot biomass × concentration) (Table 4), *B.* 414 *napus* showed the highest Cd, Al and REE contents, mainly when the plans were supplied with P and 415 shoot content was lowest in *T. aestivum*. The low P supply did not significantly affect the shoot REE 416 content of *T. aestivum*, *L. cosentinii*, *C. arietinum* and *B. napus*. However, in *L. albus* and *P. sativum*, 417 LREE and HREE contents were 40–46% (*P. sativum*) and 58–60% (*L. albus*) lower at low P supply. 418 Moreover, in *L. albus* Al, Cd contents were 48% and 71% lower. Shoot Al contents in *B. napus* was 419 47%, lower and Cd content in *T. aestivum* was 43% lower when the P supply was low.



Fig. 3 Concentrations of trace elements in shoots (left) and roots (right) of split-root plants treated without phosphorus (low P) or with 100 μ M P at the root half growing in quartz sand. LREE = sum of La – Eu, HREE = sum of Gd – Lu plus Y (means ± sd, n = 5). Differences between the P treatments were identified by t-tests with Bonferroni correction. In shoots, asterisks indicate significant differences between P treatments and means with the same capital letters were not significantly different (identified by ANOVA and Tukey's HSD post-hoc test) among plant species within a P-treatment at α = 5%. Capital letters indicate differences among plant species within a specific root half and P treatment. Lowercase

428 letters denote differences between P treatments within a species and the root side. Additionally, for roots,

429 asterisks indicate significant differences between root sides within a specific P treatment ($\alpha = 5\%$)

430

Table 4 Contents of non-essential elements in shoots of six species cultivated under addition of $100 \,\mu$ M P (P+) or no P (0P) (means ± sd; n = 5). Capital letters denote differences among the species within a P treatment. Differences in element contents between species within a P treatment were identified by ANOVA followed by Tukey's HSD post-hoc test. Differences in element contents in a species between P treatments were identified by t-tests with Bonferroni correction

			Shoot element contents				
Species	Treatment	Al	Cd	LREE	HREE		
		μg	ng	μg	μg		
Triticum	P0	$14 \pm 3B$	$13 \pm 4B$	$0.11\pm0.02C$	$0.03\pm0.01C$		
	P100	$20 \pm 12B$	$23 \pm 5BC$	$0.14\pm0.08C$	$0.04\pm0.02C$		
	p-value	0.36	0.05	0.52	0.31		
Brassica napus	P0	$68 \pm 10A$	$526 \pm 125 A$	$3.4 \pm 0.9 A$	$0.44 \pm 0.15 A$		
	P100	$128 \pm 62A$	$451 \pm 121A$	$4.3 \pm 1.0 A$	$0.64 \pm 0.18 A$		
	p-value	0.03	0.36	0.19	0.12		
Pisum sativum	P0	$59 \pm 36 AB$	$20\pm7B$	$1.24 \pm 0.33B$	$0.21\pm0.04B$		
	P100	$55 \pm 32 \text{AB}$	$24 \pm 5BC$	$2.05\pm0.34B$	$0.39\pm0.05B$		
	p-value	0.35	0.41	0.02	< 0.01		
Cicer arietinum	P0	$31 \pm 15B$	$9\pm4B$	$0.46 \pm 0.24 BC$	$0.10\pm0.06BC$		
	P100	$76 \pm 59 AB$	$10 \pm 3C$	$0.70 \pm 0.29 \mathrm{C}$	$0.16 \pm 0.08C$		
	p-value	0.17	0.72	0.18	0.21		
Lupinus albus	P0	$56 \pm 18 AB$	$23\pm9B$	$0.77\pm0.39B$	$0.18\pm0.09B$		
	P100	$107 \pm 56A$	$79\pm55B$	$1.82\pm0.55B$	$0.45\pm0.12B$		
	p-value	0.06	0.04	0.01	0.04		
Lupinus	P0	$22 \pm 14B$	$23 \pm 10B$	$0.26 \pm 0.16C$	$0.05 \pm 0.02C$		
	P100	$33 \pm 22AB$	$26 \pm 9BC$	$0.17\pm0.07C$	$0.06 \pm 0.04C$		
	p-value	0.42	0.65	0.25	0.58		
Species	P0	< 0.01	< 0.001	< 0.001	< 0.001		
	P100	< 0.01	< 0.001	< 0.001	< 0.001		

436

437 *Root accumulation of non-essential elements*

438

All investigated species showed significantly higher [LREE] and [HREE] in roots growing in quartz
sand, irrespective of the P treatment (Fig. 3). Similarly, quartz sand roots of *L. albus* and *L. cosentinii*exhibited higher [Al] and [Cd]. Considering the different P supplies, quartz sand roots of *T. aestivum*, *C. arietinum*, *L. albus* and *L. cosentinii* did not show differences in their Al, Cd, LREE and HREE
concentrations. However, in *B. napus*, the concentrations of all elements were 102% (Cd), 208% (Al),
275% (LREE) and 248% (HREE) higher in P-deficient roots than in roots supplied with P. In *P. sativum*,
P deficiency also increased [Cd] by 89% and decreased [HREE] by 38% but it did not affect [Al] and

[LREE]. In the corresponding mixed sand roots, P-supply did not alter [Al] in any of the investigated
species and [Cd] was unchanged in *T. aestivum* and *P. sativum*. However, P-deficient *B. napus*, *C. arietinum*, *L. albus* and *L. cosentinii* showed higher [Cd]. Additionally, low P supply increased [LREE]
and [HREE] in *C. arietinum* (80% and 59% increase) and *B. napus* (66% and 69% increase) and [LREE],
but not [HREE] in *L. albus* (30% increase). In contrast, in *T. aestivum*, [LREE] and [HREE] were 25%
and 20% lower at low P supply.

452 Considering data from both root parts and P treatments, the calculated LREE/HREE ratios of L. albus 453 and C. arietinum were substantially higher than those in the other species (on average 4.9–5.0 times) 454 and the lowest ratios were found in L. cosentinii (LREE/HREE = 3.6 ± 0.7). The LREE/HREE ratios 455 were higher in roots grown in quartz sand than in those in mixed sand, except for T. aestivum and P. 456 sativum. In T. aestivum, the ratios were higher in roots grown in mixed sand of P-supplied plants than 457 in corresponding roots grown in quartz sand but without differences between the P+ and P0 treatments 458 (Fig. 3). In contrast, in *P. sativum*, adding P to the quartz sand decreased the ratio from 5.0 to 4.3. 459 Similarly, in L. albus, P addition decreased the LREE/HREE ratios in both root halves (0.7 units in 460 quartz sand and 0.5 units in mixed sand).

461 Considering the root element contents (Table 4), P-supplied quartz sand roots of C. arietinum and P. 462 sativum accumulated the greatest amounts of REE and T. aestivum and L. cosentinii the lowest. In the 463 other root half growing in mixed sand, there were no differences in element contents between the species, 464 except for T. aestivum and C. arietinum, which showed a 3-4 times greater Cd content than B. napus, P. 465 sativum and L. cosentinii did. A low P supply at the root half in quartz sand did not change the contents 466 of Al and Cd in any of the investigated species, neither in quartz sand nor in mixed sand roots. However, 467 in roots grown in quartz sand, LREE and HREE contents were 45% lower in *P. sativum*. Conversely, in 468 mixed sand roots, a low P supply did not change LREE and HREE contents in P. sativum and did not 469 affect LREE in the other species. However, in *B. napus*, the low P supply tended to increase the content 470 of HREE by 83%, while in *L. cosentinii* the HREE content decreased by 44%.

471 **Table 5** Contents of light rare earth elements (LREE), heavy rare earth elements (HREE) in roots of six species cultivated under split-root conditions on two sand types,

- 472 quartz sand and mixed sand, respectively (means \pm sd; n = 5). The plants received 100 μ M P (P+) or no P (0P) in quartz sand. Capital letters denote differences among the
- 473 plant species within a P-treatment, and lowercase letters denote differences between the root halves

		Root half with P	supply (quartz san	d)		Root half without	t P supply (mixed	sand)	
Species	P-supply	LREE µg	HREE µg	Cd ng	Al µg	LREE µg	HREE μg	Cd ng	Al µg
T. aestivum	P0 P100 p-value	$\begin{array}{c} 0.57 \pm 0.23B \\ 0.81 \pm 0.42C \\ 0.28 \end{array}$	0.13 ± 0.05 $0.19 \pm 0.09BC$ 0.26	6.4 ± 3.5b 9.4 ± 5.3bBC 0.32	$\begin{array}{c} 23\pm8b\\ 34\pm14B\\ 0.18\end{array}$	$\begin{array}{c} 0.56 \pm 0.08B \\ 0.83 \pm 0.74 \\ 0.18 \end{array}$	$0.13 \pm 0.03 AB$ 0.17 ± 0.12 0.54	$\begin{array}{c} 34\pm 6aA\\ 36\pm 23aA\\ 0.81 \end{array}$	106 ± 32aA 126 ± 124 0.87
B. napus	P0 P100 p-value	$\begin{array}{c} 1.44 \pm 1.32 AB \\ 1.01 \pm 0.59 aBC \\ 0.53 \end{array}$	$\begin{array}{c} 0.30 \pm 0.26 \\ 0.23 \pm 0.15 aBC \\ 0.34 \end{array}$	$5.2 \pm 4.1b$ $7.6 \pm 2.4B$ 0.82	24 ± 20 21 ± 10B 0.34	$\begin{array}{c} 0.42 \pm 0.15B \\ 0.26 \pm 0.08b \\ 0.82 \end{array}$	$\begin{array}{c} 0.11 \pm 0.04 AB \\ 0.06 \pm 0.02b \\ 0.07 \end{array}$	13 ± 2Ba 8 ± 2B 0.84	$24 \pm 9B$ 15 ± 5 0.11
P. sativum	P0 P100 p-value	$1.7 \pm 1.0aAB$ $3.1 \pm 0.2aAB$ 0.04	$\begin{array}{c} 0.34 \pm 0.21a \\ 0.62 \pm 0.05aAB \\ 0.06 \end{array}$	$18 \pm 9 \\ 14 \pm 1BC \\ 0.47$	38 ± 20 55 ± 8AB 0.15	$\begin{array}{c} 0.53 \pm 0.27 bBC \\ 0.51 \pm 0.17 b \\ 0.51 \end{array}$	$\begin{array}{c} 0.13 \pm \! 0.07 bAB \\ 0.11 \pm 0.05 b \\ 0.74 \end{array}$	$\begin{array}{c} 17\pm10AB\\ 15\pm8AB\\ 0.74\end{array}$	$59 \pm 27AB$ 64 ± 28 0.80
C. arietinum	P0 P100 p-value	4.6 ± 4.1A 4.7 ± 1.1aA 0.96	$\begin{array}{c} 0.84 \pm 0.76 \\ 0.86 \pm 0.23 aA \\ 0.97 \end{array}$	$\begin{array}{c} 15\pm 6b\\ 18\pm 4B\\ 0.38\end{array}$	62 ± 49 50 ± 27AB 0.68	$\begin{array}{c} 1.17 \pm 0.55 A \\ 0.94 \pm 0.62 b \\ 0.68 \end{array}$	$\begin{array}{c} 0.25 \pm 0.11 A \\ 0.22 \pm 0.13 b \\ 0.73 \end{array}$	$\begin{array}{c} 29\pm8Aa\\ 31\pm10A\\ 0.81 \end{array}$	$82 \pm 35AB$ 91 ± 64 0.78
L. albus	P0 P100 p-value	$\begin{array}{c} 1.8 \pm 1.2 aAB \\ 2.2 \pm 0.6 aB \\ 0.53 \end{array}$	$\begin{array}{c} 0.32 \pm 0.21 \\ 0.44 \pm 0.11 aB \\ 0.26 \end{array}$	$\begin{array}{c} 40\pm36\\ 64\pm8aA\\ 0.14\end{array}$	59 ± 44 75 ± 19aA 0.41	$\begin{array}{c} 0.65 \pm 0.16 bAB \\ 0.76 \pm 0.32 b \\ 0.41 \end{array}$	$\begin{array}{c} 0.15 \pm 0.03 A \\ 0.13 \pm 0.02 b \\ 0.54 \end{array}$	$\begin{array}{c} 24\pm 6A\\ 21\pm 9bAB\\ 0.53\end{array}$	$51 \pm 7AB \\ 48 \pm 14b \\ 0.50$
L. cosentinii	P0 P100 p-value	$\begin{array}{c} 0.86 {\pm}~ 0.39 a AB \\ 0.79 {\pm}~ 0.29 a C \\ 0.76 \end{array}$	$\begin{array}{c} 0.22 \pm 0.11a \\ 0.19 \pm 0.07aC \\ 0.72 \end{array}$	$13 \pm 10 \\ 8 \pm 5BC \\ 0.52$	$31 \pm 15 \\ 32 \pm 26B \\ 0.94$	$\begin{array}{c} 0.15 \pm 0.08 bC \\ 0.30 \pm 0.17 b \\ 0.19 \end{array}$	$\begin{array}{c} 0.05 \pm 0.02 bB \\ 0.09 \pm 0.04 b \\ 0.07 \end{array}$	$\begin{array}{c} 6.0 \pm 1.9B \\ 8.9 \pm 3.7B \\ 0.86 \end{array}$	$17 \pm 10B$ 30 ± 13 0.21
Species p-value	P0 P100	0.03 <0.001	0.06 <0.001	0.06 <0.001	0.35 <0.01	< 0.01 0.12	0.04 0.08	<0.001 <0.01	< 0.01 0.48

477 The substrate-normalized [REE] calculated for shoots treated with different P levels showed clear 478 differences among the species and partly depended on the treatment with P (Fig. 4). In all plant species, 479 the normalized REE concentrations were <<1, and the pattern was generally similar among the species 480 tested with curves downward from left to right showing LREE-enrichment and HREE-depletion.



482 Fig. 4 Substrate-normalized REE patterns (calculated by dividing the shoot concentrations by the
483 average total element concentrations of the sand substrates) in A) *Triticum aestivum*, B) *Brassica napus*,
484 C) *Pisum sativum*, D) *Cicer arietinum*, E) *Lupinus albus* and F) *L. cosentinii* treated with 100 μM P
485 (P+) or no P (0P)

In *B. napus* and *P. sativum*, the normalized [LREE] relative to [HREE] was much higher than those in *T. aestivum*, *C. arietinum*, *L. albus* and *L. cosentinii* showing LREE/HREE > 1. Moreover, *B. napus* and

P. sativum exhibited steeper curves than *T. aestivum*, *C. arietinum*, *L. albus* and *L. cosentinii* did.
Concerning the effects of P addition, *C. arietinum* did not show any differences in the REE pattern
between P-supplied and P-deficient plants. When the plants were cultivated under P-deficient
conditions, *T. aestivum* and *B. napus* displayed higher normalized [REE], particularly [LREE] and
middle-mass [REE] (Gd–Er). In contrast, *P. sativum*, *L. albus* and *L. cosentinii*, showed significantly
higher [LREE] and [HREE] when the plants were supplied with P in the nutrient solution.

495

496 **Discussion**

497

498 Plant growth, root biomass and nutritional status

499

500 In the present experiment, cultivation of plants with split roots growing in different sand types allowed 501 us to control the P supply to one root half without influencing REE availability directly through the 502 precipitation of Al and REE with phosphate in the presence of P. The treatment with low P supply 503 showed less production of shoot and total plant biomass of T. aestivum, B. napus, and L. albus, whereas 504 there was no effect on C. arietinum, P. sativum and L. cosentinii (Fig. 1). The latter species showed 505 virtually unchanged shoot [P] following P addition (Table 2). Shoot [P] did not exceed the concentration 506 that is adequate for crop growth of 2 mg P g^{-1} dry weight (Marschner 1995), except in *T. aestivum*. This 507 was unexpected, given that the plants received a high supply of P (100 μ M P as KH₂PO₄) in the nutrient 508 solution. It is possible that a considerable amount of P sorbed onto Al and Fe oxides and hydroxides 509 of the acidic quartz sand. Additionally, after five weeks of plant growth, all plants entered the 510 reproduction phase, so P remobilization to the seeds may have contributed to the low shoot [P] (El 511 Mazlouzi et al. 2020). Shoot [Fe] and [Mn] were largely unchanged at the low P supply. However, lower 512 concentrations of Mn and Fe in P-deficient T. aestivum and P. sativum (Table 2) might indicate a reduced 513 uptake and/or translocation capacity (Fan et al. 2021). Root [P] was higher in all species in the high P-514 half. This was not only observed in the root half in contact with the nutrient solution, but also in the 515 other root half, grown in mixed sand of T. aestivum, C. arietinum and L. albus, and to some extent in 516 roots of L. cosentinii (Fig. 3). Conversely, [P] of roots grown in mixed sand of B. napus and P. sativum 517 were unaffected. Indeed, the [P] was highly influenced by root growth. However, all plants developed 518 more root biomass in the mixed sand. Hence, these findings indicate that the plants allocated a large 519 portion of P absorbed in quartz sand to the other root half growing in mixed sand. The increased root 520 mass ratios of T. aestivum, B. napus and L. albus in P0 treatments (Fig. 1) indicate a relatively increased 521 allocation of dry matter to roots and adjustment of root growth to a low P supply (de Bang et al. 2020). 522 This growth adjustment is determined by the overall nutrient status of the plants (Robinson 1996), and, 523 therefore, might explain the high biomass allocation in *T. aestivum*, which showed the largest differences 524 in shoot [P] resulting from differences in P supply. In contrast, L. cosentinii, C. arietinum and P. sativum 525 did not respond to differences in P supply with altered root mass ratios. These species presumably relied 526 more heavily on chemical changes in the rhizosphere than on more extensive root systems (Pearse et al. 527 2006). In the present experiment, the mixed sand (roots without P supply) was characterized by a higher 528 pH and higher P availability (Table 1). Therefore, in the present experiment, resource allocation must 529 be considered not only between shoot and roots but also between the different root halves (Fig. 1), 530 allowing us to explore the capacity to respond to nutrient availability by plasticity in root development. 531 Indeed, when P supply was low in quartz sand (P0), all species (except L. cosentinii) developed more 532 extensive roots in the mixed sand where the plants were exposed to conditions that allowed them to 533 acquire more nutrients. The P0 treatment reduced the root growth of *B. napus* in quartz sand, but did not 534 affect the root mass of other species at this root side.

535 When the P supply was higher at the root side in quartz sand, the root mass of L. albus was unaffected 536 in mixed sand, but B. napus showed a lower root mass. In contrast, L. cosentinii and C. arietinum had a 537 higher root mass in mixed sand (Fig. 1) when the plants were supplied with P to the roots in quartz sand. 538 This suggests that under P deficiency, the phosphophile B. napus mainly relies on readily-available P 539 sources and effectively adjusts its root growth to the compartment where P can be most easily acquired. 540 In contrast, C. arietinum, L. albus and especially L. cosentinii appeared to follow a more conservative 541 strategy and sustained root development in the mixed sand with higher total nutrient concentrations, 542 increasing the chance to maintain P and micronutrient supply through changes in rhizosphere chemistry.

After five weeks of plant growth, all species tested had entered the reproductive phase and started flowering. So the carboxylate release observed in the present study may not necessarily characterize the plant's nutrient-acquisition efficiency, because carboxylate release typically declines when plants enter the reproductive stage (Mimmo et al. 2011). However, the observed exudation rates among P-supplied and P-deficient plants can be used to characterize the species' general response to the P status (Fig. 2). In this study, the amount of carboxylates released from the different root halves per unit time (Fig. 2B) integrates root mass and carboxylate release per unit mass (Fig. 2C). They characterize the species'

ability to chemically influence the root environments. In contrast, exudation rates per unit of time androot mass characterize the physiological response to environmental conditions.

555 *Triticum aestivum*, *B. napus*, *P. sativum* and *C. arietinum* did not show differences in rhizosphere pH in 556 response to P supply (Fig. 2D); however, the rhizosheath pH was lowest in *P. sativum* and *C. arietinum* 557 (Fig. 2D), highlighting the capacity of these species to acidify the rhizosphere irrespective of P supply 558 (Pearse et al. 2006). In contrast, *L. albus*, and *L. cosentinii* strongly acidified the rhizosphere when P 559 was lacking in the nutrient solution, especially in the mixed sand.

560 It is generally assumed that the response of plants to nutrient deficiency is determined by the overall 561 nutrient status of the plant, as demonstrated for lupins and some Proteaceae species (Shane et al. 2003a, 562 2003b; Wang et al. 2013). However, In C. arietinum, the production and release of carboxylates appears 563 to be independent of plant P status (Wouterlood et al. 2004) and B. napus and T. aestivum typically show 564 slow and declining carboxylate release under P-deficient conditions (Pearse et al. 2006). Consistently, 565 in the present study, P deficiency increased the carboxylate release of the lupins, reduced carboxylate 566 release in B. napus and P. sativum, but did not affect the amount of carboxylates released in C. arietinum 567 and T. aestivum (Fig. 2A, B).

Besides changes in amounts, the composition of root exudates is an integral factor determining P-mining efficiency (Jones 1998; Lambers 2022). Despite the lower carboxylate release of *B. napus* at a low P supply, *B. napus* released the greatest amounts of carboxylates, mainly malate. Compared with dicarboxylates (malate) released by *T. aestivum*, *B. napus* and *P. sativum*, citrate, a tricarboxylate which was the dominantly carboxylate released by *C. arietinum*, *L. albus* and *L. cosentinii* (Fig. 2A) forms 573 more stable complexes with soil cations and consequently is more efficient at releasing P and 574 micronutrients by complexation and ligand exchange reactions (Jones 1998). Moreover, when P supply 575 was low in the quartz sand substrate, all species responded with decreased amounts of carboxylate 576 release at this root side (Fig. 2B) which was primarily due to reduced root mass as a consequence of P 577 starvation (Fig. 1). In contrast, carboxylate-exudation rates were unaffected in P-starved roots of L. albus 578 and L. cosentinii and these species showed an up-regulation of carboxylate release in roots in mixed 579 sand (Fig. 2B, C) attributing to these species' ability to respond to a low P supply with adjustment of 580 root activity and rhizosphere chemistry.

581

582 Accumulation of non-essential elements related to P-supply and carboxylate release

583

584 A low P supply may affect the accumulation of non-essential elements through i) altered plant growth 585 and thus an enrichment per unit biomass, ii) altered uptake and translocation when uptake is mediated 586 by nutrient transporters that are affected by the growth-limiting nutrient, and iii) altered solubility and 587 chemical speciation in the rhizosphere determining the accessibility for transport mechanisms. If altered 588 solubility is involved, when the availability is limited by mobility in soil, any increase in solubility 589 following changes in chemical speciation will ultimately increase diffusion towards the root and the 590 probability of the element entering the root. Conversely, when the mobility of elements is high(er), 591 changes in the chemical speciation from the ionic form to a metal-organic complex may decrease 592 availability through exclusion at the site of uptake (Barber and Lee 1974). In the present experiment, all 593 plants altered the rhizosheath pH and released carboxylates depending on species and P supply (Fig. 2). 594 The sand substrates contained the elements in sparingly soluble forms (Table 1). Less than 0.1% of Cd, 595 Fe, Mn, and Al were present in mobile forms (Fraction 1). In contrast, the solubility of REE was 596 somewhat higher, especially in the quartz sand (Table 1). Nonetheless, all species contained detectable 597 concentrations of all elements with high variability among the species tested (Fig. 1). Aluminum and 598 REE showed a similar behavior in the shoots, consistent with the literature (Liu et al. 2021; Fehlauer et 599 al. 2022). In *B. napus*, high shoot and low root [Cd] can be primarily explained by the efficient influx 600 and transport of Cd from roots to shoots (Selvam and Wong 2009).

601 Concerning the effect of P supply, the REE concentrations in shoots and roots responded more 602 sensitively than those of Al and Cd, given that four out of six species showed significant differences in 603 [LREE] and [HREE] following a reduction of P supply (Fig. 3). Of these species, C. arietinum and T. 604 aestivum did not respond to altered element accumulation and showed a relatively flat normalized REE 605 pattern with a slight decrease in HREE accumulation (Gd-Lu) (Fig. 4). These species did not respond 606 to a low P supply with altered carboxylate release (Fig. 2). The higher LREE and Al concentrations in 607 roots in mixed sand of P-deficient C. arietinum (Fig. 3) corresponded with less root biomass (Fig. 1). 608 Enrichment could largely explain this in the roots which led to unchanged element contents in the plants 609 (Table 3). Thus, the higher concentrations of Al and LREE in shoots and roots of P-deficient T. aestivum 610 (Fig. 3) were accompanied by lower biomass production (Fig. 1) and unchanged element amounts 611 accumulated in the plant compartments (Tables 3; 4).

612 Similar to T. aestivum, the shoot biomass of B. napus was lower as a consequence of lower P supply 613 (Fig. 1) but without changes in Cd, Al and HREE concentrations (Fig. 3), whereas LREE concentrations 614 were significantly higher (Fig. 3) and Al, LREE, and HREE contents were less. (Table 3). Additionally, 615 in P-deficient plants, total carboxylate release was less (Fig. 2), suggesting that the element pattern in 616 shoots resulted from less element uptake in concert with a preferential root-shoot transfer of LREE 617 relative to HREE and LREE accumulation in shoots. HREE form more stable complexes with low-618 molecular-weight organic anions, for instance, citrate, during long-distance transport in the xylem (Ma 619 and Hiradate 2000; Yuan et al. 2017). However, based on the higher charge density, HREE are 620 preferentially sorbed onto cell walls during radial transport and form more stable complexes with 621 metabolites released into the rhizosphere. Given that REEs are predominantly taken up in ionic form 622 through Ca, K, and Na channels (Han et al. 2005), carboxylates and other chelating compounds would 623 alter the chemical speciation, and hence the uptake and accumulation of REE, including the ratio of 624 LREE/HREE (Wiche et al. 2017b). Element exclusion through extracellular complexation has been 625 studied in detail for Al in Al-resistant species (Zheng et al. 1998; Ma et al. 2001; Kochian et al. 2004) 626 and Cd in L. albus (Römer et al. 2000). For a specific carboxylate (e.g., citrate), the complex stabilities 627 decrease in the order HREE > LREE > Al > Cd (Byrne and Li 1995; Martell et al. 2004), while for a 628 given element (e.g., La), the complex stabilities decrease in the order citrate > malate > acetate (Fig. 5).



Fig. 5 Stability constants (T = 25°C or 20°C) for REE complexation with organic acid anions. Data
from Martell et al. (2004)

629

633 Han et al. (2005) demonstrated that organic acids promote the uptake of La by barley, but the effect of 634 the acid decreased in the order acetic acid > malic acid > citric acid, which can be explained mainly by 635 decreased sorption of La onto the apoplast in the presence of the acid anion but a reduced uptake with 636 increasing complex stability (Han et al. 2005). In the present experiment, B. napus released large 637 quantities of malate (Fig. 2), a dicarboxylate with a lower complexation constant (La: log K 4.37) 638 compared with that of citrate (La: log K 7.63). Nonetheless, the large quantities released should favour 639 complex formation and element exclusion, which might also explain the lower total REE concentrations 640 in B. napus than in P. sativum. Pisum sativum released much smaller amounts of dicarboxylates but 641 strongly acidified the rhizosphere (Fig. 2D) and mobilized the elements in plant-available (ionic) forms 642 (Cao et al. 2001; Wiche et al. 2017b). Slight differences in the complexation behavior between LREE 643 and HREE might have influenced the LREE accumulation in this species at a low P supply (Figs 3; 4). 644 Indeed, P-deficient roots exposed to quartz sand with higher mobility of REE (Table 1) showed higher 645 concentrations of Al, LREE and HREE but did not affect net root sorption (Table 3) with lower 646 carboxylate release (Fig. 2). Conversely, P-deficient roots in mixed sand released greater amounts of 647 carboxylates (Fig. 2B) and showed higher concentrations (Fig. 3) and element contents (Table 3), most 648 likely through increased element dissolution followed by decreased internal element transport. This 649 contention is supported by the responses in *P. sativum*, *L. albus* and *L. cosentinii*. *Pisum sativum* strongly 650 acidified the rhizosheath in both root parts, irrespective of P supply, and released only small amounts of 651 carboxylates, mainly malate (Fig. 3). A reduction in P supply did not change shoot and root biomass 652 (Fig. 1). Still, it decreased the concentrations and contents (Fig. 3) of LREE, HREE, Fe and Mn with 653 higher LREE/HREE ratios in P-deficient plants. Conversely, in shoots of L. albus and L. cosentinii, the 654 concentrations and contents of LREE, HREE and Cd declined (Fig. 3) at a low P-supply which was 655 accompanied by greater exudation of citrate (Fig. 2). Although in L. cosentinii, this effect was somewhat 656 less pronounced than in L. albus, in L. cosentinii P-deficient plants displayed significantly lower 657 LREE/HREE ratios indicating a higher HREE translocation relative to LREE when P-supply was low. 658 In contrast, P-deficient roots of L. albus showed higher LREE/HREE ratios, irrespective of the root half, 659 while in L. cosentinii, the LREE/HREE ratios in roots were unaffected. This can be primarily explained 660 by the strong acidification of the rhizosphere of *L. cosentinii*, shifting the carboxylic acid: carboxylate 661 ratio towards the acid form (Pearse et al. 2006), preventing complex formation and favouring uptake of 662 LREE in L. albus but not in L. cosentinii. In the latter species the presence of carboxylates might have 663 increased the release and uptake of HREE from sparingly-available element forms from the HREE-664 enriched mixed sand (Table 1).

665

666 Conclusion

667

668 We demonstrated that plant P status influenced the accumulation of the non-essential elements Cd, Al, 669 and REE, beyond the commonly recognized mechanism of REE-phosphate precipitation in roots. Plants 670 that strongly acidified the rhizosphere and released small quantities of dicarboxylates accumulated the 671 highest concentrations of REE. Conversely, modest rhizosphere acidification and large amounts of 672 carboxylates were associated with a significantly lower accumulation of REE. Phosphophile species or 673 plants that do not respond to P deficiency (B. napus, T. aestivum, C. arietinum) with increased 674 carboxylate release accumulated REE to higher concentrations when P supply was low, which was 675 explained largely by reduced growth and thus enrichment of the elements in the plant biomass. 676 Additionally, in these species REE-phosphate precipitation might have contributed to a lower REE 677 accumulation in P-supplied plants. In contrast, plants that released more tricarboxylates under conditions 678 of P deficiency accumulated more REE when the P supply was high and carboxylate release was low. 679 The proposed mechanism involves the mobilization of the elements in the rhizosphere through 680 carboxylate and proton release, pH-dependent formation of REE-carboxylate complexes with complex 681 stabilities depending on the amount and composition of carboxylates with HREE-complexes > LREE-682 complexes and exclusion of the complexes during uptake, radial transport and/or translocation. This 683 suggests a functional overlap of carboxylate-based belowground traits related to P nutrition and 684 exclusion of REE, which otherwise might become toxic in REE-enriched growth environments. The 685 relationship between plant nutrition and REE accumulation could also explain the large variability in 686 REE accumulation among different plant species and plant individuals growing in the same soil. The 687 proposed model provides a mechanistic explanation for the REE-hyperaccumulation in Proteaceae (Van 688 der Ent et al. 2023) and highlights the potential of leaf REE signatures to characterize plant species 689 regarding their P-acquisition strategy through changes in rhizosphere chemistry following an ionomic 690 approach.

691

692 Acknowledgements

693

694	These studies have been carried out in the framework of a habilitation fellowship granted to OW by the
695	German Academic Exchange Service (DAAD).

696

697 **References**

698

699	Arnon DI, Stout PR (1939)	The essentiality of	certain elements	in minute quar	itity for	plants v	with
-----	---------------------------	---------------------	------------------	----------------	-----------	----------	------

700 special reference to copper. Plant Physiol 14:371-375.

701 https://dx.doi.org/10.1104/pp.14.2.371

702

703	Barber DA, Lee RB (1974) The effect	of micro-organisms	on the absorption	of manganese by plants.
-----	-------------------------	--------------	--------------------	-------------------	-------------------------

704 New Phytol 73:97-106. <u>https://doi.org/10.1111/j.1469-8137.1974.tb04610.x</u>

706	Brioschi L, Steinmann M, Lucot E, Pierret MC, Stille P, Prunier J, Badot PM (2013) Transfer of rare
707	earth elements (REE) from natural soil to plant systems: implications for the environmental
708	availability of anthropogenic REE. Plant Soil 366, 143–163. https://doi.org/10.1007/s11104-012-1407-
709	<u>0</u>
710	
711	Byrne RH, Li B (1995) Comparative complexation behavior of the rare earths. Geochim Cosmochim
712	Acta, 59:4575-4589. https://doi.org/10.1016/0016-7037(95)00303-7
713	
714	
715	Cao X, Chen Y, Wang X, Deng X (2001) Effects of redox potential and pH value on the release of rare
716	earth elements from soil. Chemosphere 44:655-661. https://doi.org/10.1016/S0045-6535(00)00492-6
717	
718	Davranche M, Gruau G, Dia A, Bouhnik-Le Coz M, Marsac R, Pédrot M, Pourret O (2017) Rare earth
719	elements in wetlands, trace elements in waterlogged soils and sSediments. Taylor & Francis
720	Group/CRC Press, pp. 135-162.
721	
722	de Bang TC, Husted S, Laursen KH, Persson DP, Schjoering JK (2020) The molecular-physiological
723	functions of mineral macronutrients and their consequences for deficiency symptoms in plants. New
724	Phytol 229:2446-2469. https://doi.org/10.1111/nph.17074
725	
726	de Tombeur F, Laliberté E, Lambers H, Faucon M-P, Zemunik G, Turner BL, Cornelis J-T, Mahy G
727	(2021) Plants sustain the terrestrial silicon cycle during ecosystem retrogression. Science 369:1245-
728	1248. https://doi.org/10.1126/science.abc0393
729	
730	Diatloff E, Asher CJ, Smith, FW (1999) The effects of rare earth elements on the growth and nutrition
731	of plants. Rare Earths '98, Mater Sci Forum 315:354-360.
732	https://doi.org/10.4028/www.scientific.net/MSF.315-317.354
733	

734	El Mazlouzi M, Morel C, Chesseron C, Robert T, Mollier A (2020) Contribution of external and internal
735	phosphorus sources to grain P loading in durum wheat (Triticum durum L.) grown under contrasting P
736	levels. Front Plant Sci. https://doi.org/10.3389/fpls.2020.00870
737	
738	Fan X, Zhou X, Chen H, Tang M, Xie X (2021) Cross-talks between macro- and micronutrient uptake
739	and signaling in plants. Front Plant Sci. https://doi.org/10.3389/fpls.2021.663477
740	
741	Fehlauer T, Colline B, Angletti B, Negahi MM, Dentant C, Chaurand P, Lallemand C, Levard C, Rose
742	J (2022) Multiscale imaging on Saxifraga paniculata provides new insights into yttrium uptake by
743	plants. Sci Rep 18268. https://doi.org/10.1038/s41598-022-23107-x
744	
745	Han F, Shan X-Q, Zhang J, Xie Y-N, Pei Z-G, Zhang S-Z, Zhu Y-G, Wen B (2005) Organic acids
746	promote the uptake of lanthanum by barley roots. New Phytol 165:481-492.
747	https://doi.org/10.1111/j.1469-8137.2004.01256.x
748	
749	
750	
751	Honvault N, Houben D, Firmin S, Meglouli H, Laruelle F, Fontaine J, Lounès-Hadj Sahraoui A, Coutu
752	A, Lambers H, Faucon MP (2021a) Interactions between below-ground traits and rhizosheath fungal
753	and bacterial communities for phosphorus acquisition. Funct Ecol 35:1603-1619.
754	https://doi.org/10.1111/1365-2435.13823
755	
756	Honvault N, Houben D, Nobile C, Firmin S, Lambers H, Faucon M-P (2021) Tradeoffs among
757	phosphorus-acquisition root traits of crop species for agroecological intensification. Plant Soil 461:
758	137-150. https://doi.org/10.1007/s11104-020-04584-3
759	
760	Jones DL (1998) Organic acids in the rhizosphere – a critical review. Plant Soil 205:25-44.
761	

- Khan AM, Bakar NKA, Bakar AFA, Ashraf MA (2016) Chemical speciation and bioavailability of rare
 earth elements (REEs) in the ecosystem: a review. Environ Sci Pol Res 24:22764-22789.
 https://doi.org/10.1007/s11356-016-7427-1
- 765

Kochian LV, Hoekenga OA and Piñeros MA (2004) How do crop plants tolerate acid soils? Mechanisms
of aluminum tolerance and phosphorous efficiency. Annu Rev Plant Biol 55:459-493.
10.1146/annurev.arplant.55.031903.141655.

- 769
- Kovarikova M, Tomášková I, Soudek P (2019) Rare earth elements in plants. <u>Biol Plant 63</u>:20-32.
 https://doi.org/10.32615/bp.2019.003
- 772

Krachler M, Mohl C, Emons H, Shotyk W (2002) Analytical procedures for the determination of
selected trace elements in peat and plant samples by inductively coupled plasma mass spectrometry.

775 Spectrochim Acta B Spectrosc 57(8):1277-1289. https://doi.org/10.1016/S0584-8547(02)00068-X

776

Krasilnikoff G, Gahoonia T, Nielsen NE (2003) Variation in phosphorus uptake efficiency by genotypes
of cowpea (*Vigna unguiculata*) due to differences in root and root hair length and induced rhizosphere

779 processes. Plant Soil 251:83-91. https://doi.org/10.1023/A:1022934213879

780

Tambers H (2022) Phosphorus acquisition and utilization in plants. Annu Rev Plant Biol 73:1.1-1.26.

782 https://doi.org/10.1146/annurev-arplant-102720-125738

783

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

788	Lambers H, Clements JC, Nelson MN (2013) How a phosphorus-acquisition strategy based on
789	carboxylate exudation powers the success and agronomic potential of lupines (Lupinus, Fabaceae). Am
790	J Bot 100: 263-288. https://doi.org/10.3732/ajb.1200474
791	
792	Li T, Kaercher S, Roesky PW (2014) Synthesis, structure and reactivity of rare-earth metal complexes
793	containing anionic phosphorus ligands. Chem Soc Rev 43:42-57. https://doi.org/10.1039/C3CS60163C
794	
795	Liu C, Ding T-X, Liu W-S, Tang Y-T, Qiu R-L (2022) Phosphorus mediated rhizosphere mobilization
796	and apoplast precipitation regulate rare earth element accumulation in Phytolacca americana. Plant Soil
797	240. https://doi.org/10.1007/s11104-022-05743-4
798	
799	Liu C, Liu W-S, van der Ent A, Morel JL, Zheng HX, Wang G-B, Tang Y-T, Qiu R-L (2021)
800	Simultaneous hyperaccumulation of rare earth elements, manganese and aluminum in Phytolacca
801	americana in response to soil properties. Chemosphere 282, 131096
802	https://doi.org/10.1016/j.chemosphere.2021.131096.
802 803	https://doi.org/10.1016/j.chemosphere.2021.131096.
802 803 804	https://doi.org/10.1016/j.chemosphere.2021.131096. Ma JF, Ryan PR and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of
802803804805	https://doi.org/10.1016/j.chemosphere.2021.131096. Ma JF, Ryan PR and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273-278. https://doi.org/10.1016/S1360-1385(01)01961-6
 802 803 804 805 806 	https://doi.org/10.1016/j.chemosphere.2021.131096. Ma JF, Ryan PR and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273-278. https://doi.org/10.1016/S1360-1385(01)01961-6
 802 803 804 805 806 807 	 https://doi.org/10.1016/j.chemosphere.2021.131096. Ma JF, Ryan PR and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273-278. https://doi.org/10.1016/S1360-1385(01)01961-6 Ma JF, Hirdate F (2000) Form of aluminium for uptake and translocation in buckwheat (<i>Fagopyrum</i>)
 802 803 804 805 806 807 808 	 https://doi.org/10.1016/j.chemosphere.2021.131096. Ma JF, Ryan PR and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273-278. https://doi.org/10.1016/S1360-1385(01)01961-6 Ma JF, Hirdate F (2000) Form of aluminium for uptake and translocation in buckwheat (<i>Fagopyrum esculentum</i> Moench). Planta 211:355-360. https://doi.org/10.1007/s004250000292
 802 803 804 805 806 807 808 809 	 https://doi.org/10.1016/j.chemosphere.2021.131096. Ma JF, Ryan PR and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273-278. https://doi.org/10.1016/S1360-1385(01)01961-6 Ma JF, Hirdate F (2000) Form of aluminium for uptake and translocation in buckwheat (<i>Fagopyrum esculentum</i> Moench). Planta 211:355-360. https://doi.org/10.1007/s004250000292
 802 803 804 805 806 807 808 809 810 	 https://doi.org/10.1016/j.chemosphere.2021.131096. Ma JF, Ryan PR and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273-278. https://doi.org/10.1016/S1360-1385(01)01961-6 Ma JF, Hirdate F (2000) Form of aluminium for uptake and translocation in buckwheat (<i>Fagopyrum esculentum</i> Moench). Planta 211:355-360. https://doi.org/10.1007/s004250000292 McLennan SM (2001) Relationships between the trace element composition of sedimentary rocks and
 802 803 804 805 806 807 808 809 810 811 	 https://doi.org/10.1016/j.chemosphere.2021.131096. Ma JF, Ryan PR and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273-278. https://doi.org/10.1016/S1360-1385(01)01961-6 Ma JF, Hirdate F (2000) Form of aluminium for uptake and translocation in buckwheat (<i>Fagopyrum esculentum</i> Moench). Planta 211:355-360. https://doi.org/10.1007/s004250000292 McLennan SM (2001) Relationships between the trace element composition of sedimentary rocks and upper continental crust. Geochem Geophys 2. https://doi.org/10.1029/2000GC000109
 802 803 804 805 806 807 808 809 810 811 812 	 https://doi.org/10.1016/j.chemosphere.2021.131096. Ma JF, Ryan PR and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273-278. https://doi.org/10.1016/S1360-1385(01)01961-6 Ma JF, Hirdate F (2000) Form of aluminium for uptake and translocation in buckwheat (<i>Fagopyrum esculentum</i> Moench). Planta 211:355-360. https://doi.org/10.1007/s004250000292 McLennan SM (2001) Relationships between the trace element composition of sedimentary rocks and upper continental crust. Geochem Geophys 2. https://doi.org/10.1029/2000GC000109
 802 803 804 805 806 807 808 809 810 811 812 813 	 https://doi.org/10.1016/j.chemosphere.2021.131096. Ma JF, Ryan PR and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273-278. https://doi.org/10.1016/S1360-1385(01)01961-6 Ma JF, Hirdate F (2000) Form of aluminium for uptake and translocation in buckwheat (<i>Fagopyrum esculentum</i> Moench). Planta 211:355-360. https://doi.org/10.1007/s004250000292 McLennan SM (2001) Relationships between the trace element composition of sedimentary rocks and upper continental crust. Geochem Geophys 2. https://doi.org/10.1029/2000GC000109 Marschner H (1995) Mineral nutrition of higher plants. Academic Press, London.
 802 803 804 805 806 807 808 809 810 811 812 813 814 	 https://doi.org/10.1016/j.chemosphere.2021.131096. Ma JF, Ryan PR and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273-278. https://doi.org/10.1016/S1360-1385(01)01961-6 Ma JF, Hirdate F (2000) Form of aluminium for uptake and translocation in buckwheat (<i>Fagopyrum esculentum</i> Moench). Planta 211:355-360. https://doi.org/10.1007/s004250000292 McLennan SM (2001) Relationships between the trace element composition of sedimentary rocks and upper continental crust. Geochem Geophys 2. https://doi.org/10.1029/2000GC000109 Marschner H (1995) Mineral nutrition of higher plants. Academic Press, London.

815 Martell AE, Smith RM, Motekaitis RJ (2004). NIST Critically Selected Stability Constants of Metal

817

- 818 Mimmo T, Hann S, Jaitz L, Cesco S, Gessa CE, Puschenreiter M (2011) Time and substrate dependent
- 819 exudation of carboxylates by Lupinus albus L. and Brassica napus L. Plant Physiol Biochem 49:1272-
- 820 1278. https://doi.org/10.1016/j.plaphy.2011.08.012

821

Monei N, Hitch M, Heim J, Pourret O, Heilmeier H, Wiche O (2022) Effect of substrate properties and
P-supply on facilitating the uptake of rare earth elements (REE) in mixed-culture cropping systems of *Hordeum vulgare, Lupinus albus* and *Lupinus angustifolius*. Environ Sci Poll Res 29, 57172–57189.
https://doi.org/10.1007/s11356-022-19775-x

826

- Neumann G, Römheld V (2001) The release of root exudates as affected by the plant's physiological
 status. In: Pinton R, Varanini Z, Nannipieri P (eds) The Rhizosphere Biochemistry and organic
 substances at the soil–plant interface. Marcel Dekker AG, Basel, Switzerland.
- 830
- Neumann G, Massonneau A, Langlade N, Dinkelaker B, Hengeler C, Römheld V, Martinoia E (2000)
 Physiological aspects of cluster root function and development in phosphorus-deficient white lupin
 (*Lupinus albus* L.). Ann Bot 85:909-919. https://doi.org/10.1006/anbo.2000.1135

834

Pang J, Ruchi B, Zhao H, Bansal R, Bohuon E, Lambers H, Ryan MH, Ranathunge K, Siddique KMH
(2018) The carboxylate-releasing phosphorus-mobilising strategy could be proxied by foliar manganese
concentration in a large set of chickpea germplasm under low phosphorus supply. New Phytol 219:518529. https://doi.org/10.1111/nph.15200

- 840 Pearse SJ, Veneklaas EJ, Cawthray G, Bolland MDA, Lambers H (2006) Carboxylate composition of
- root exudates does not relate consistently to a crop species' ability to use phosphorus from aluminium,

^{816 452} Complexes, Database 46, Version 8, NIST, Gaithersburg.

- 842 iron or calcium phosphate sources. New Phytol 173:181-190. https://doi.org/10.1111/j.1469843 8137.2006.01897.x
- 844
- 845 Pourret O, van der Ent A, Hursthouse A, Irawan DE, Liu H, Wiche O (2022) The 'europium anomaly'
- 846 in plants: facts and fiction. Plant Soil 476:721-728. https://doi.org/10.1007/s11104-021-05210-6
- 847
- 848 Pourret O, Davranche M, Gruau G, Dia A (2007) Rare earth elements complexation with humic acid.
- 849 Chem Geol 243:128-141. https://doi.org/10.1016/j.chemgeo.2007.05.018
- 850
- 851 Reimann C, Siewers U, Tarvainen T, Bityukova L, Eriksson J, Giucis A, Gregorauskiene V, Lukashev
- 852 VK, Matinian NN, Pasieczna A (2003) Agricultural soils in northern Europe. Schweizerbart, Stuttgart.
 853
- Robinson D (1996) Variation, co-ordination and compensation in root systems in relation to soil
 variability. Plant Soil 187:57-66. https://doi.org/10.1007/BF00011657
- 856
- 857 Römer W, Kang D-K, Egle K, Gerke J, Keller H (2000) The acquisition of cadmium by *Lupinus albus*
- 858 L., Lupinus angustifolius L., and Lolium multiflorum Lam. J Plant Nutr Soil Sci 163:623-628.
- 859 https://doi.org/10.1002/1522-2624(200012)163:6<623::AID-JPLN623>3.0.CO;2-C
- 860
- Saiz-Fernandez I, Cerny M, Skalak J, Brzobohaty B (2021) Split-root systems: detailed methodology,
 alternative applications, and implications at leaf proteome level. Plant Methods 17.
 https://dx.doi.org/10.1186/s13007-020-00706-1
- 864
- Schwabe R, Dittrich C, Kadner J, Senges CHR, Bandow JE, Tischler D, Schlömann M, Levicán G,
 Wiche O (2021) Secondary metabolites released by the rhizosphere bacteria *Arthrobacter oxydans* and *Kocuria rosea* enhance plant availability and soil–plant transfer of germanium (Ge) and rare earth
 elements (REEs). Chemosphere 285, 131466. https://doi.org/10.1016/j.chemosphere.2021.131466
- 869

Selvam A, Wong JW-C (2009) Cadmium uptake potential of Brassica napus cocropped with Brassica										
parachinensis and Zea mays. J Hazard Mater 167:170-178.										
https://doi.org/10.1016/j.jhazmat.2008.12.103										
Shane MW, Lambers H (2005) Cluster roots: a curiosity in context. Plant Soil 274:101-125.										
10.007/s11104-004-2725-7										
Shane MW, De Vos M, De Roock S, Lambers H (2003a) Shoot P status regulates cluster-root growth										
and citrate exudation in Lupinus albus grown with a divided root system. Plant Cell Environ 26:265-										
273. https://doi.org/10.1046/j.1365-3040.2003.00957.x.										
Shane M W, De Vos M, De Roock S, Cawthray G R and Lambers H (2003b) Effects of external										
phosphorus supply on internal phosphorus concentration and the initiation, growth and exudation of										
cluster roots in <i>Hakea prostrata</i> R.Br. Plant Soil 248:209-219.										
https://doi.org/10.1023/A:1022320416038.										
Tyler G (2004) Rare earth elements in soil and plant systems - A review. Plant Soil 267:191-206.										
https://doi.org/10.1007/s11104-005-4888-2										
van der Ent A, Nkrumah PN, Purwadi I, Erskine PD (2023) Rare earth element (hyper)accumulation in										
some Proteaceae from Queensland, Australia. Plant Soil. https://doi.org/10.1007/s11104-022-05805-7										
Van Laak M, Klingenberg U, Peiter E, Reitz T, Zimmer D, Buczko U (2018) The equivalence of the										
calcium-acetate-lactate and double-lactate extraction methods to assess soil phosphorus fertility. J Plant										
Nutr Soil Sci 181:795-801. https://doi.org/10.1002/jpln.201700366										

- 896 Wang X, Pearse SJ, Lambers H (2013) Cluster-root formation and carboxylate release in three Lupinus
- species as dependent on phosphorus supply, internal phosphorus concentration and relative growth rate.
- 898 Ann Bot 112: 1449-1459. https://doi.org/10.1093/aob/mct210
- 899
- Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation
 (phytoremediation) of soils. Plant Soil 321:385-408. https://doi.org/10.1007/s11104-008-9686-1
- 902
- 903 Wiche O, Zertani V, Hentschel W, Achtziger R, Midula P (2017a) Germanium and rare earth elements
- 904 in topsoil and soil-grown plants on different land use types in the mining area of Freiberg (Germany). J
- 905 Geochem Explor 175:120-129. https://doi.org/10.1016/j.gexplo.2017.01.008
- 906
- 907 Wiche O, Tischler D, Fauser C, Lodemann J, Heilmeier H (2017b) Effects of citric acid and the 908 siderophore desferrioxamine B (DFO-B) on the mobility of germanium and rare earth elements in soil 909 J Phytoremediation 19:746-754. and uptake in **Phalaris** arundinacea. Int 910 https://doi.org/10.1080/15226514.2017.1284752
- 911
- Wiche O, Kummer N-A, Heilmeier H (2016a). Interspecific root interactions between white lupin and
 barley enhance the uptake of rare earth elements (REEs) and nutrients in shoots of barley. Plant Soil
 402:235-245. https://doi.org/10.1007/s11104-016-2797-1
- 915
- Wiche O, Székely B, Kummer N-A, Moschner C, Heilmeier H (2016b) Effects of intercropping of oat
 (*Avena sativa* L.) with white lupin (*Lupinus albus* L.) on the mobility of target elements for
 phytoremediation and phytomining in soil solution. Int J Phytoremediation 18:900-907.
 https://doi.org/10.1080/15226514.2016.1156635
- 920

Wouterlood M, Cawthray GR, Scanlon T, Lambers H, Veneklaas EJ. (2004) Carboxylate concentrations
in the rhizosphere of lateral roots of chickpea (*Cicer arietinum*) increase during plant development, but

923	are not correlated with phosphorus status of soil or plants. New Phytol 162: 745-753.								
924	https://doi.org/10.1111/j.1469-8137.2004.01070.x								
925									
926	Wyttenbach A, Furrer V, Schleppi P, Tobler L (1998) Rare earth elements in soil and in soil-grown								
927	plants. Plant Soil 199:267-273. https://doi.org/10.1023/A:1004331826160								
928									
929	Yuan M, Guo M-N, Liu W-S, Liu C, van der Ent A, Morel JL, Huot H, Zhao W-Y, Wei X-G, Qiu R-								
930	L, Tang Y-T (2017) The accumulation and fractionation of Rare Earth Elements in hydroponically								
931	grown Phytolacca americana L. Plant Soil 421:67-82. https://doi.org/10.1007/s11104-017-3426-3								
932									
933	Zheng SJ, Ma JF, Matsumoto H (1998) High aluminum resistance in buckwheat: I. Al-induced specific								
934	secretion of oxalic acid from root tips. Plant Phys 117:745-751. https://doi.org/10.1104/pp.117.3.745								
935									
936	Statements and Declarations								
937									
938	Funding								
939									
940	OW was supported by a habilitation fellowship granted by the German Academic Exchange Service								
941	(grant number 91758065).								
942									
943	Competing Interests								
944									
945	The authors have no relevant financial or non-financial interests to disclose.								
946									
947	Author Contributions								
948									
949	All authors contributed to the study conception and design. Material preparation, data collection and								
950	analysis were performed by Oliver Wiche, Christine Dittrich, Nthati Monei and Juliane Heim. The first								

951	draft of the	manuscript was	written by	Oliver	Wiche	and all	authors	commented	on	versions	of 1	the
<i>JJI</i>	ununt of the	manuseript was	written by	Onvoi	W ICHC	una un	autions	commented	on	versions	UI I	une

952 manuscript. All authors read and approved the final manuscript.

954 Data Availability

- All data obtained during the experiment are contained in the manuscript.

-

-

979 Captions

980

Table 1 Total element concentrations and distribution of elements in exchangeable (F1), acid-soluble (F2), oxidizable (F3) and moderately-reducible (F4) fractions ($\mu g g^{-1}$) according to Wiche et al. (2017) determined by a sequential extraction method (mean \pm sd; n = 10). Differences in means between the two sand types are identified by t-tests with Bonferroni correction. Means with different letters are significantly different at $\alpha = 5\%$

986

987 **Table 2** Concentration of nutrients in shoots of six plant species cultivated under split-root conditions 988 (means \pm sd; n = 5) and addition of 100 μ M P (P+) and no P in the treatment solution (0P). Capital 989 letters denote significant differences among the species within a P treatment

990

991**Table 3** Nutrient concentrations in roots of six species cultivated under split-root conditions on two sand992types, quartz sand and mixed sand (means \pm sd; n = 5). The plants received 100 μ M P (P+) or no P (0P)993in quartz sand. Capital letters denote differences among plant species within a P treatment, and994lowercase letters denote differences between the root halves for a specific element

995

Table 4 Contents of non-essential elements in shoots of six species cultivated under addition of $100 \,\mu$ M P (P+) or no P (0P) (means ± sd; n = 5). Capital letters denote differences among the species within a P treatment. Differences in element contents between species within a P treatment were identified by ANOVA followed by Tukey's HSD post-hoc test. Differences in element contents in a species between P treatments were identified by t-tests with Bonferroni correction

1001

Table 5 Contents of light rare earth elements (LREE), heavy rare earth elements (HREE) in roots of six species cultivated under split-root conditions on two sand types, quartz sand and mixed sand, respectively (means \pm sd; n = 5). The plants received 100 μ M P (P+) or no P (0P) in quartz sand. Capital letters denote differences among the plant species within a P-treatment, and lowercase letters denote differences between the root halves

1008 Fig. 1 Shoot mass and root mass (g) of the two root halves growing in quartz sand (Q) and mixed sand 1009 (M) and root mass ratio considering both root halves depending on treatment with 100 µM P (P+) and 1010 no P (0P). The bars represent means \pm se (n = 5). ANOVA identified differences among species (capital 1011 letters) following Tukey's HSD post-hoc test at $\alpha = 5\%$. Significant differences in shoot mass and root mass ratios between different P-treatments are indicated by asterisks (**p < 0.01; *p < 0.05; (*)p1012 1013 <0.1).Differences in root masses between the root halves within a P treatment are indicated with 1014 lowercase letters. Capital letters denote differences among the species within a P-treatment. Means with 1015 the same letters are not significantly different at $\alpha = 5\%$

1016

1017 Fig. 2 Total carboxylate release per plant (A), (B) carboxylate release from root halves growing in quartz 1018 sand (Q) and mixed sand (M), (C) exudation rates from the different root halves, and (D) rhizosphere 1019 pH depending on treatment of plants with 100 µM P (P+) or no P (0P) from the root half growing in 1020 quartz sand (means \pm se, n = 5). Capital letters indicate significant differences between species within a 1021 P treatment. Small letters indicate i) differences among species concerning a specific carboxylate type 1022 within a P-treatment (A), ii) differences in carboxylate release between different root halves within a P-1023 treatment and species (B, C) or iii) differences in the pH between the same root half at different P supply 1024 rates (D). Means with different letters are significantly different at $\alpha = 5\%$ identified by Tukey's HSD 1025 post-hoc test

1026

Fig. 3 Concentrations of trace elements in shoots (left) and roots (right) of split-root plants treated without phosphorus (low P) or with 100 μ M P at the root half growing in quartz sand. LREE = sum of La – Eu, HREE = sum of Gd – Lu plus Y (means ± sd, n = 5). Differences between the P treatments were identified by t-tests with Bonferroni correction. In shoots, asterisks indicate significant differences between P treatments and means with the same capital letters were not significantly different (identified by ANOVA and Tukey's HSD post-hoc test) among plant species within a P-treatment at α = 5%. Capital letters indicate differences among plant species within a specific root half and P treatment. Lowercase 1034 letters denote differences between P treatments within a species and the root side. Additionally, for roots, 1035 asterisks indicate significant differences between root sides within a specific P treatment ($\alpha = 5\%$)

1036

- Fig. 4 Substrate-normalized REE patterns (calculated by dividing the shoot concentrations by the
 average total element concentrations of the sand substrates) in A) *Triticum aestivum*, B) *Brassica napus*,
 C) *Pisum sativum*, D) *Cicer arietinum*, E) *Lupinus albus* and F) *L. cosentinii* treated with 100 μM P
 (P+) or no P (0P)
- 1041
- 1042 Fig. 5 Stability constants ($T = 25^{\circ}C$ or 20°C) for REE complexation with organic acid anions. Data
- 1043 from Martell et al. (2004)