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

3

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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.

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35 Keywords: element exclusion, rhizosphere chemistry, complexation, carboxylates

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57 **Introduction**

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59 Root carboxylate release is an essential plant strategy to access sparingly-available soil nutrients,
60 especially inorganic phosphate (Pi), iron (Fe), and manganese (Mn) (Shane and Lambers 2005; Lambers
61 2022). Plants have adapted to conditions of heterogeneously distributed and sparsely-available soil
62 resources and evolved strategies to influence the properties of the soil surrounding their roots
63 (rhizosphere) to create an environment more conducive for nutrient acquisition (rhizosheath). In addition
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-impooverished 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).

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

85 (REEs) (Wenzel 2009, Wiche et al. 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 $\mu\text{g g}^{-1}$ (Ce), 30 $\mu\text{g g}^{-1}$ (La) and 28 $\mu\text{g g}^{-1}$ (Nd) to 0.3 $\mu\text{g g}^{-1}$ (Lu) (McLennan 2001;
93 Davranche et al. 2017). As a unique feature in this group, all 16 REEs exhibit ionic radii similar to Ca^{2+} ;
94 however, under most pedologically-relevant conditions, REEs form trivalent cations (Wytenbach 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 REE^{3+} -
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 Al^{3+} (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)
131 were filled with quartz sand (0.1–0.4 mm grain size, 1500 kg m⁻³), while the other half was filled with a
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 ±
136 0.3 mg kg⁻¹ P. In both sand types, the total element concentrations were similar (Table 1); however, the
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.

151 **Table 1** Total element concentrations and distribution of elements in exchangeable (F1), acid-soluble (F2), oxidizable (F3) and moderately-reducible (F4) fractions
 152 ($\mu\text{g g}^{-1}$) 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
 153 identified by t-tests with Bonferroni correction. Means with different letters are significantly different at $\alpha = 5\%$

Substrate	Fraction	P	Mn	Fe	Al	Cd	LREE	HREE
Quartz sand	Total	358 \pm 50a	466 \pm 245	1773 \pm 635	5279 \pm 1134	1.44 \pm 0.56	12.3 \pm 3.4	3.6 \pm 0.9
	1	<0.5	0.4 \pm 0.1b	0.8 \pm 0.2	2.1 \pm 0.6a	<0.05	0.40 \pm 0.03a	0.10 \pm 0.01
	2	<0.5	0.14 \pm 0.12b	6.5 \pm 1.3b	18 \pm 2a	<0.05	0.15 \pm 0.03b	0.05 \pm 0.01b
	3	123 \pm 10b	0.19 \pm 0.10b	7.9 \pm 2.0b	4.9 \pm 0.6b	<0.05	0.10 \pm 0.04b	0.21 \pm 0.09b
	4	26.2 \pm 2.9	0.78 \pm 0.09b	89 \pm 14	37.8 \pm 2.6a	0.09 \pm 0.01b	0.60 \pm 0.06b	0.17 \pm 0.03b
	5	<0.5b	0.64 \pm 0.14b	71 \pm 31b	73.7 \pm 7.2b	0.09 \pm 0.01b	0.81 \pm 0.13b	0.14 \pm 0.03b
Mixed sand	Total	281 \pm 36b	334 \pm 125	2174 \pm 748	5617 \pm 1990	1.38 \pm 0.34	13.8 \pm 1.9	5.0 \pm 1.1
	1	<0.5	2.2 \pm 0.5a	1.0 \pm 0.4	1.4 \pm 0.2b	<0.05	0.33 \pm 0.04b	0.09 \pm 0.01
	2	<0.5	0.71 \pm 0.42a	10.3 \pm 2.4a	13 \pm 3b	<0.05	0.19 \pm 0.03a	0.06 \pm 0.01a
	3	165 \pm 16a	1.87 \pm 1.12a	12.3 \pm 2.7a	7.6 \pm 1.1a	<0.05	0.18 \pm 0.05a	0.41 \pm 0.17a
	4	23.8 \pm 2.6	2.17 \pm 0.86a	95 \pm 19	32.9 \pm 3.1b	0.11 \pm 0.01a	0.76 \pm 0.08a	0.25 \pm 0.04a
	5	8.0 \pm 1.7a	4.44 \pm 1.69a	518 \pm 149a	98.7 \pm 14.2a	0.12 \pm 0.01a	1.03 \pm 0.19a	0.24 \pm 0.06a

154

155 *Plant growth*

156

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
163 600 $\mu\text{mol m}^{-2} \text{s}^{-2}$ photosynthetically active radiation. After one week, the primary roots of *B. napus*, *P.*
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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 μM 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 CaCl_2 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 CaCl_2 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

208 The dried plant material was ground to a fine powder using a centrifugal mill equipped with a titanium
209 rotor (Retsch ZM 100) and stored in centrifuge tubes. Afterwards, microwave digestion (Ethos plus 2,
210 MLS, Leutkirch, Germany) was carried out with 0.1 g of subsample taken from the ground biomass and
211 measured in duplicate. Samples were mixed with 1.6 mL nitric acid (65% suprapure) and 0.6 mL
212 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,
215 Thermo Scientific, Dreieich, Germany) using 10 $\mu\text{g L}^{-1}$ rhodium and rhenium as internal standards.
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
221 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 $\alpha = 5\%$.

237

238 **Results**

239

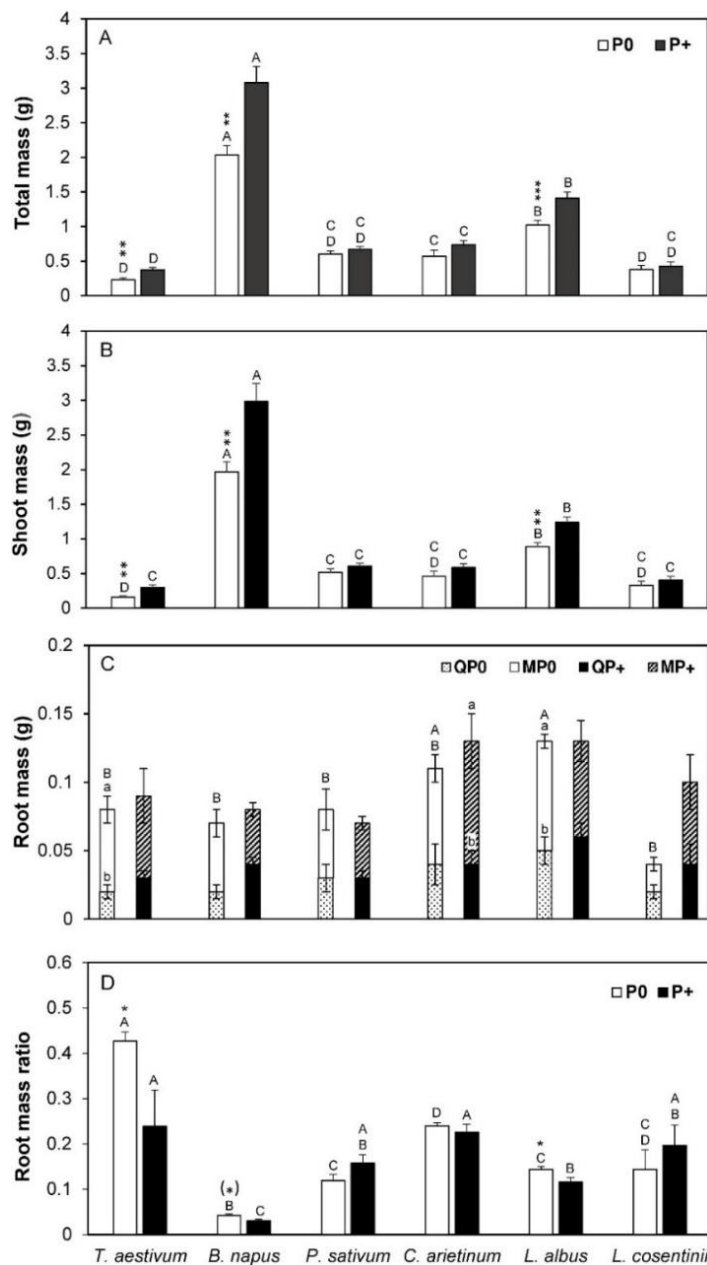
240 *Plant growth and biomass responding to P-supply*

241

242 The dry biomass varied considerably among the tested plant species (Figure 1). *Brassica napus*
243 accumulated the most biomass (3.1 g) when P was supplied, and *T. aestivum* accumulated the least
244 biomass when no P was supplied (0.2 g) (Fig. 1A). Phosphorus supply increased the total dry mass
245 (shoot and root mass) of *T. aestivum*, *B. napus*, *C. arietinum* and *L. albus* by 65%, 52%, 27%, and 56%,
246 respectively. In contrast, the total biomass of *P. sativum* and *L. cosentini* did not respond to P supply
247 (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. cosentini*, and there were no differences among *P. sativum*, *L.*
250 *cosentini* 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. cosentini*, 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. cosentini* (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. cosentini* > *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. cosentini*). 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 *cosentini*, but lowest in *B. napus* (Fig. 1D).



268

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 μ M 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; (*)p

274 <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 $\alpha = 5\%$

277

278 *Shoot nutrient accumulation*

279

280 Shoot [P] of plants watered with 100 μM P ranged from 1.21 mg g^{-1} (*B. napus*) to 2.46 mg g^{-1} (*T.*
 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
 288 P-supply. The addition of low P doses tended to decrease shoot [Mn] in *T. aestivum*, *B. napus*, and *P.*
 289 *sativum* and significantly decreased [Fe] in *P. sativum*. In contrast, shoot [Mn] in low-P plants of *L.*
 290 *albus* and *L. cosentinii* were consistently higher than those in plants that received P with the watering
 291 solution (Table 2).

292

293 **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 μM P (P+) and no P in the treatment solution (0P). Capital letters
 295 denote significant differences among the species within a P treatment

Species	P-treatment	P mg g^{-1}	Mn $\mu\text{g g}^{-1}$	Fe
<i>Triticum aestivum</i>	P0	1.06 \pm 0.05B	160 \pm 35B	122 \pm 42A
	P+	2.46 \pm 1.03A	206 \pm 29B	99 \pm 22AB
	p-value	0.02	0.06	0.32
<i>Brassica napus</i>	P0	0.98 \pm 0.06B	97 \pm 17C	39 \pm 3B
	P+	1.21 \pm 0.12B	114 \pm 13BC	45 \pm 8B
	p-value	0.05	0.22	0.14
<i>Pisum sativum</i>	P0	1.14 \pm 0.09AB	88 \pm 18BC	67 \pm 10BC
	P+	1.31 \pm 0.02B	100 \pm 36BC	97 \pm 17AB
	p-value	0.11	0.50	< 0.01
<i>Cicer arietinum</i>	P0	1.23 \pm 0.17A	63 \pm 18C	84 \pm 31BC
	P+	1.30 \pm 0.11B	53 \pm 21C	97 \pm 43AB
	p-value	0.56	0.42	0.59
<i>Lupinus albus</i>	P0	1.25 \pm 0.15A	452 \pm 96A	160 \pm 71A
	P+	1.42 \pm 0.071B	413 \pm 51A	107 \pm 30A
	p-value	0.05	0.25	0.15
<i>Lupinus cosentinii</i>	P0	1.39 \pm 0.17A	121 \pm 58BC	88 \pm 18BC
	P+	1.73 \pm 0.16A	96 \pm 38BC	87 \pm 20AB
	p-value	0.03	0.56	0.91
Species	P0	<0.001	< 0.001	< 0.01
	P+	<0.01	<0.001	< 0.01

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297

298 *Root nutrient accumulation*

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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 (0P) decreased root [P] of
303 all species by 10–25% compared with plants treated with 100 μ M 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%).

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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

Species	treatment	Root part with P supply (quartz sand)			Root part without P supply (mixed sand)		
		P mg g ⁻¹	Mn μ g g ⁻¹	Fe	P mg g ⁻¹	Mn μ g g ⁻¹	Fe
<i>T. aestivum</i>	P0	1.34 \pm 0.25aAB	103 \pm 28bA	8345 \pm 2889aA	0.93 \pm 0.13bC	175 \pm 49aA	1784 \pm 440bA
	P100	1.78 \pm 0.23aA	131 \pm 27bAB	6270 \pm 2032aA	1.15 \pm 0.09bC	252 \pm 45aA	2614 \pm 585bA
	p-value	0.02	0.14	0.13	0.01	0.06	0.04
<i>B. napus</i>	P0	1.54 \pm 0.19A	148 \pm 83A	1243 \pm 697B	1.53 \pm 0.10A	97 \pm 23B	391 \pm 97C
	P100	1.79 \pm 0.11aA	86 \pm 40AB	476 \pm 132C	1.56 \pm 0.06bA	67 \pm 21C	316 \pm 48D
	p-value	0.03	0.23	0.04	0.65	0.10	0.31
<i>P. sativum</i>	P0	1.52 \pm 0.13A	152 \pm 109AB	1692 \pm 792B	1.39 \pm 0.08A	165 \pm 71AB	1213 \pm 434AB
	P100	1.68 \pm 0.04aA	64 \pm 35B	1489 \pm 34aB	1.35 \pm 0.07bB	90 \pm 26BC	1100 \pm 89bB
	p-value	0.06	0.24	0.68	0.44	0.08	0.63
<i>C. arietinum</i>	P0	1.32 \pm 0.09aAB	129 \pm 43bA	1515 \pm 260aB	1.15 \pm 0.06bB	205 \pm 30aA	1014 \pm 82bB
	P100	1.85 \pm 0.30aA	213 \pm 126A	1909 \pm 799aB	1.21 \pm 0.07bBC	133 \pm 17B	758 \pm 171bC
	p-value	<0.01	0.20	0.38	0.15	<0.01	0.02
<i>L. albus</i>	P0	1.10 \pm 0.26B	47 \pm 8B	1325 \pm 250aB	1.08 \pm 0.04B	43 \pm 8C	632 \pm 79bB
	P100	1.35 \pm 0.08B	44 \pm 14B	919 \pm 145aB	1.31 \pm 0.17BC	36 \pm 5C	790 \pm 109bC
	p-value	0.09	0.72	0.01	0.03	0.18	0.25
<i>L. cosentinii</i>	P0	1.38 \pm 0.17A	134 \pm 23A	2691 \pm 2035B	1.20 \pm 0.04B	123 \pm 77AB	1075 \pm 537AB
	P100	1.63 \pm 0.45AB	81 \pm 53AB	2610 \pm 1567B	1.30 \pm 0.05BC	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

331
 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).

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

363

364 *Rhizosphere acidification in response to P supply*

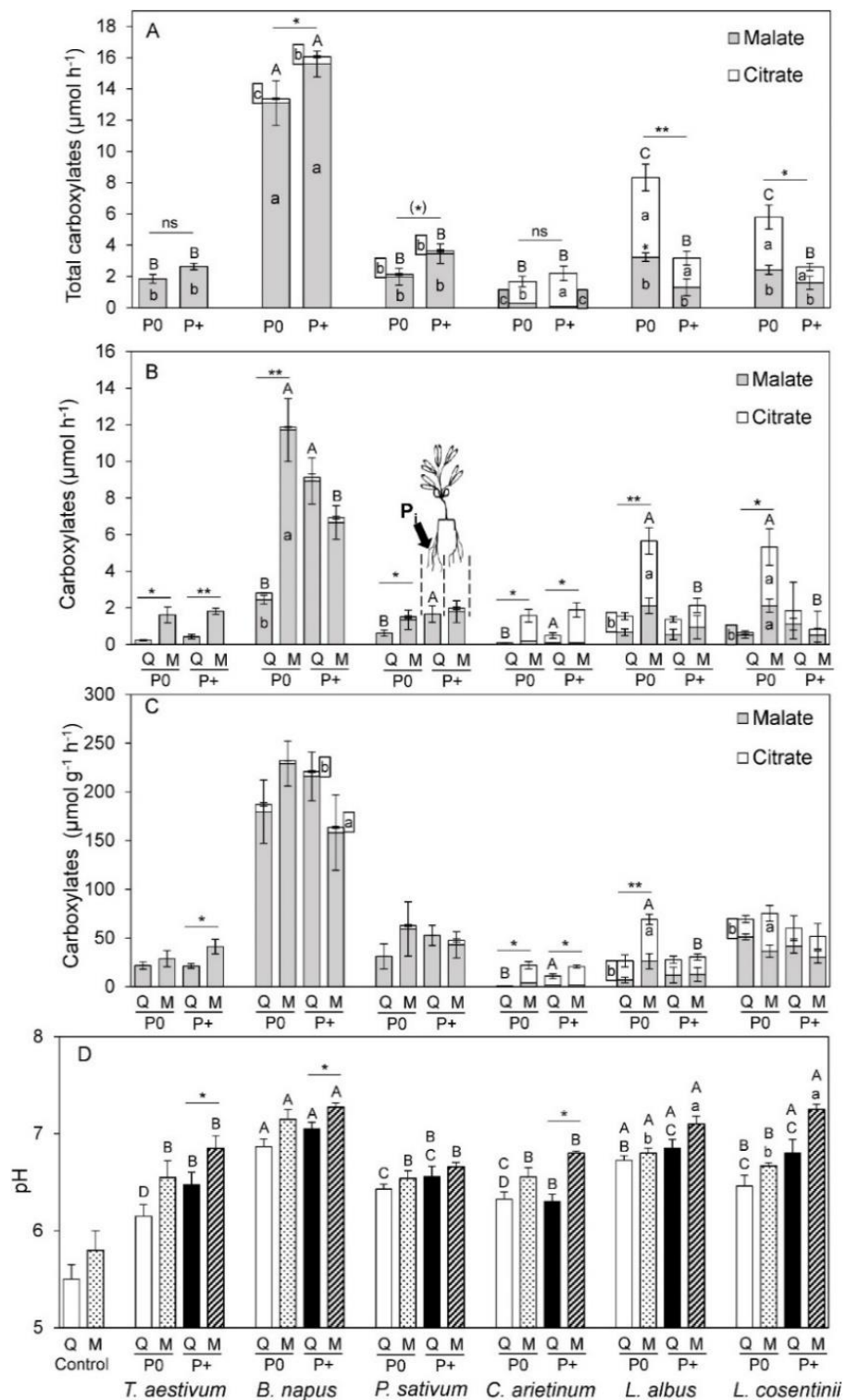
365

366 In the rhizosphere 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* ($\text{pH } 7.1 \pm 0.2$) was on average 0.5 units higher
372 than that of *C. arietinum*, *P. sativum* and *T. aestivum* ($\text{pH } 6.6 \pm 0.1$). When the P supply was low, the
373 pH in the rhizosphere 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.

376 Considering the different root halves under P+ conditions, the pH in the quartz sand rhizosphere was
377 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 ± 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)
381 higher than that of *T. aestivum*, *C. arietinum* and *P. sativum* (6.8 ± 0.1) ($\alpha = 1\%$). Here, the low P supply
382 reduced the pH in the rhizosphere of *L. albus* and *B. napus* by 0.2 units and strongly reduced the pH in
383 the rhizosphere of *L. cosentinii*, by 0.6 units.



384

385 **Fig. 2** Total carboxylate release per plant (A), (B) carboxylate release from root halves growing in quartz
 386 sand (Q) and mixed sand (M), (C) exudation rates from the different root halves, and (D) rhizosphere
 387 pH depending on treatment of plants with 100 μM P (P+) or no P (P0) from the root half growing in
 388 quartz sand (means \pm se, n = 5). Capital letters indicate significant differences between species within a
 389 P treatment. Small letters indicate i) differences among species concerning a specific carboxylate type
 390 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

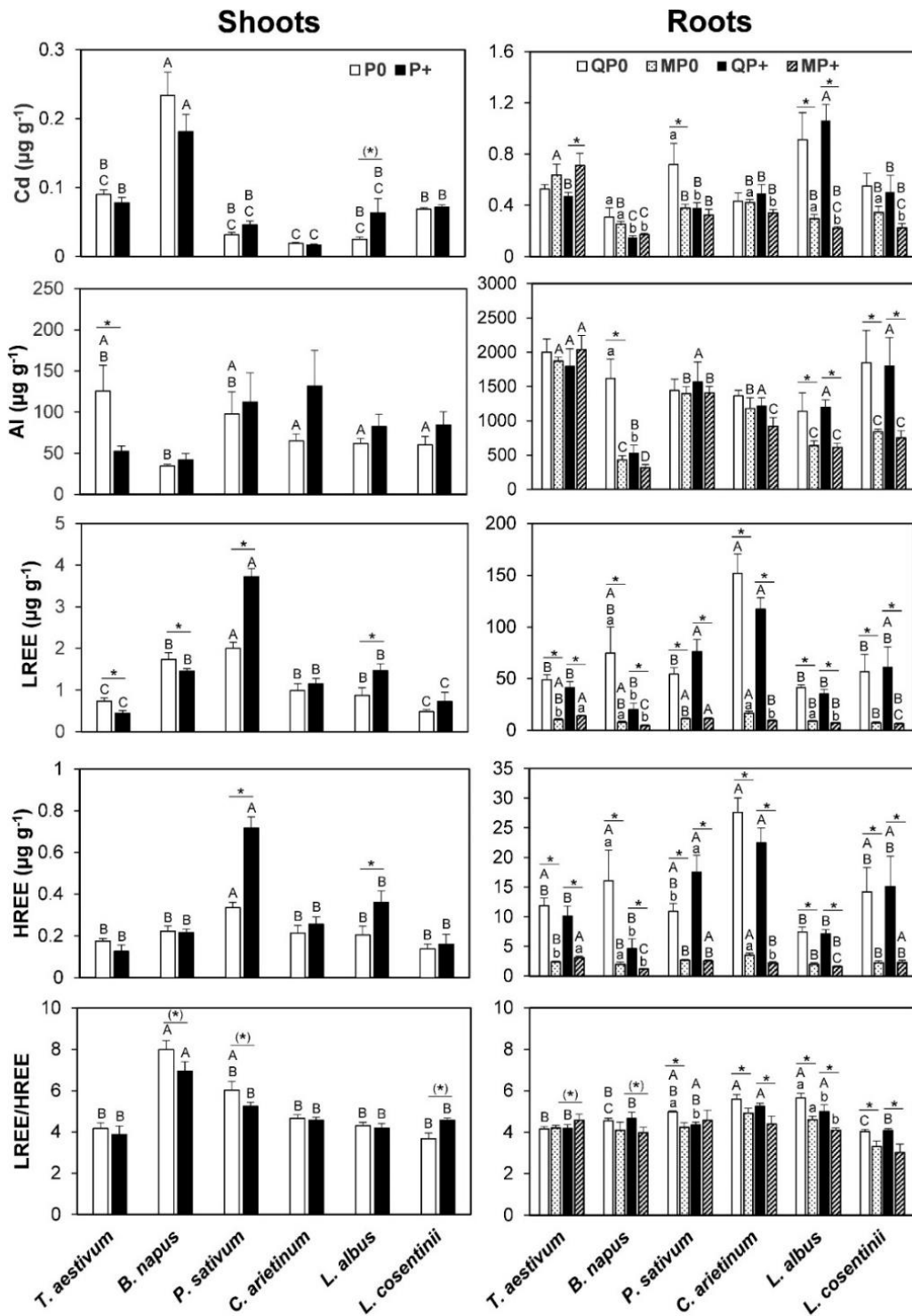
395 *Shoot accumulation of non-essential elements*

396

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

403 Phosphorus supply did not significantly affect shoot [Al] 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 \times concentration) (Table 4), *B.*
414 *napus* showed the highest Cd, Al and REE contents, mainly when the plants 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.



420
 421 **Fig. 3** Concentrations of trace elements in shoots (left) and roots (right) of split-root plants treated
 422 without phosphorus (low P) or with 100 μM P at the root half growing in quartz sand. LREE = sum of
 423 La – Eu, HREE = sum of Gd – Lu plus Y (means \pm sd, n = 5). Differences between the P treatments
 424 were identified by t-tests with Bonferroni correction. In shoots, asterisks indicate significant differences
 425 between P treatments and means with the same capital letters were not significantly different (identified
 426 by ANOVA and Tukey's HSD post-hoc test) among plant species within a P-treatment at $\alpha = 5\%$. Capital
 427 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
 431 **Table 4** Contents of non-essential elements in shoots of six species cultivated under addition of 100 μM
 432 P (P+) or no P (OP) (means \pm sd; n = 5). Capital letters denote differences among the species within a P
 433 treatment. Differences in element contents between species within a P treatment were identified by
 434 ANOVA followed by Tukey's HSD post-hoc test. Differences in element contents in a species between
 435 P treatments were identified by t-tests with Bonferroni correction

Species	Treatment	Shoot element contents			
		Al μg	Cd ng	LREE μg	HREE μg
<i>Triticum</i>	P0	14 \pm 3B	13 \pm 4B	0.11 \pm 0.02C	0.03 \pm 0.01C
	P100	20 \pm 12B	23 \pm 5BC	0.14 \pm 0.08C	0.04 \pm 0.02C
	p-value	0.36	0.05	0.52	0.31
<i>Brassica napus</i>	P0	68 \pm 10A	526 \pm 125A	3.4 \pm 0.9A	0.44 \pm 0.15A
	P100	128 \pm 62A	451 \pm 121A	4.3 \pm 1.0A	0.64 \pm 0.18A
	p-value	0.03	0.36	0.19	0.12
<i>Pisum sativum</i>	P0	59 \pm 36AB	20 \pm 7B	1.24 \pm 0.33B	0.21 \pm 0.04B
	P100	55 \pm 32AB	24 \pm 5BC	2.05 \pm 0.34B	0.39 \pm 0.05B
	p-value	0.35	0.41	0.02	<0.01
<i>Cicer arietinum</i>	P0	31 \pm 15B	9 \pm 4B	0.46 \pm 0.24BC	0.10 \pm 0.06BC
	P100	76 \pm 59AB	10 \pm 3C	0.70 \pm 0.29C	0.16 \pm 0.08C
	p-value	0.17	0.72	0.18	0.21
<i>Lupinus albus</i>	P0	56 \pm 18AB	23 \pm 9B	0.77 \pm 0.39B	0.18 \pm 0.09B
	P100	107 \pm 56A	79 \pm 55B	1.82 \pm 0.55B	0.45 \pm 0.12B
	p-value	0.06	0.04	0.01	0.04
<i>Lupinus</i>	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 \pm 0.07C	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

439 All investigated species showed significantly higher [LREE] and [HREE] in roots growing in quartz
 440 sand, irrespective of the P treatment (Fig. 3). Similarly, quartz sand roots of *L. albus* and *L. cosentinii*
 441 exhibited higher [Al] and [Cd]. Considering the different P supplies, quartz sand roots of *T. aestivum*,
 442 *C. arietinum*, *L. albus* and *L. cosentinii* did not show differences in their Al, Cd, LREE and HREE
 443 concentrations. However, in *B. napus*, the concentrations of all elements were 102% (Cd), 208% (Al),
 444 275% (LREE) and 248% (HREE) higher in P-deficient roots than in roots supplied with P. In *P. sativum*,
 445 P deficiency also increased [Cd] by 89% and decreased [HREE] by 38% but it did not affect [Al] and

446 [LREE]. In the corresponding mixed sand roots, P-supply did not alter [Al] in any of the investigated
447 species and [Cd] was unchanged in *T. aestivum* and *P. sativum*. However, P-deficient *B. napus*, *C.*
448 *arietinum*, *L. albus* and *L. cosentinii* showed higher [Cd]. Additionally, low P supply increased [LREE]
449 and [HREE] in *C. arietinum* (80% and 59% increase) and *B. napus* (66% and 69% increase) and [LREE],
450 but not [HREE] in *L. albus* (30% increase). In contrast, in *T. aestivum*, [LREE] and [HREE] were 25%
451 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

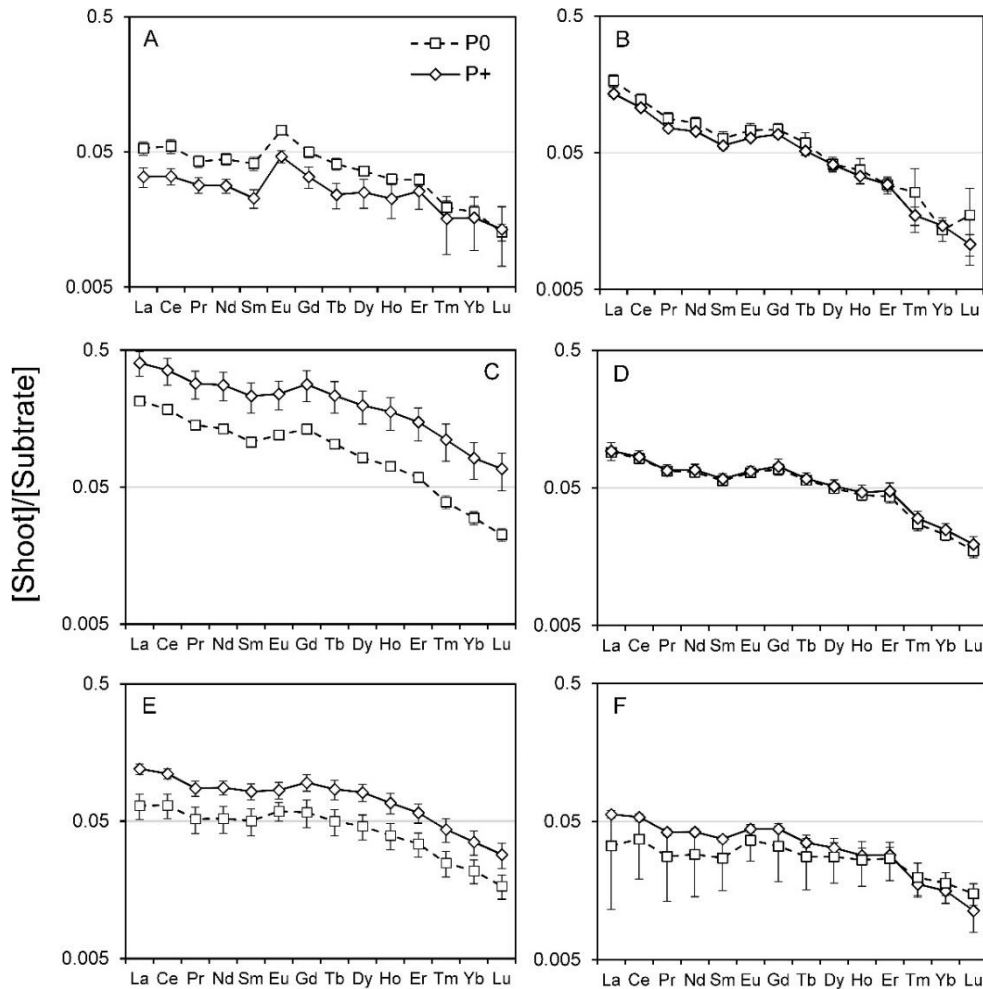
Species	P-supply	Root half with P supply (quartz sand)				Root half without P supply (mixed sand)			
		LREE μ g	HREE μ g	Cd ng	Al μ g	LREE μ g	HREE μ g	Cd ng	Al μ g
<i>T. aestivum</i>	P0	0.57 \pm 0.23B	0.13 \pm 0.05	6.4 \pm 3.5b	23 \pm 8b	0.56 \pm 0.08B	0.13 \pm 0.03AB	34 \pm 6aA	106 \pm 32aA
	P100	0.81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

474

475 Normalized REE pattern in shoots and responses to P supply

476

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 $\ll 1$, and the pattern was generally similar among the species
480 tested with curves downward from left to right showing LREE-enrichment and HREE-depletion.



481

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 μ M P
485 (P+) or no P (0P)

486

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

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

543

544 *Modifications of rhizosphere chemistry in response to P supply*

545

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

568 Besides changes in amounts, the composition of root exudates is an integral factor determining P-mining
569 efficiency (Jones 1998; Lambers 2022). Despite the lower carboxylate release of *B. napus* at a low P
570 supply, *B. napus* released the greatest amounts of carboxylates, mainly malate. Compared with
571 dicarboxylates (malate) released by *T. aestivum*, *B. napus* and *P. sativum*, citrate, a tricarboxylate which
572 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

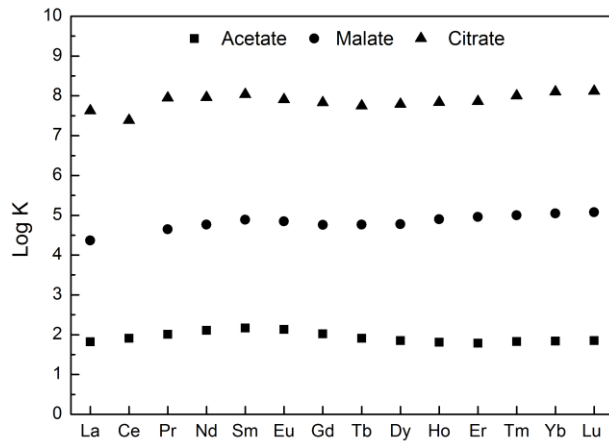
582 *Accumulation of non-essential elements related to P-supply and carboxylate release*

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; Fehlaue 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).



629

630 **Fig. 5** Stability constants ($T = 25^{\circ}\text{C}$ or 20°C) for REE complexation with organic acid anions. Data
 631 from Martell et al. (2004)

632

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 ionic
690 approach.

691

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693

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696

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937

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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, Nthathi Monei and Juliane Heim. The first

951 draft of the manuscript was written by Oliver Wiche and all authors commented on versions of the
952 manuscript. All authors read and approved the final manuscript.

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954 **Data Availability**

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

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979 **Captions**

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981 **Table 1** Total element concentrations and distribution of elements in exchangeable (F1), acid-soluble
982 (F2), oxidizable (F3) and moderately-reducible (F4) fractions ($\mu\text{g g}^{-1}$) according to Wiche et al. (2017)
983 determined by a sequential extraction method (mean \pm sd; n = 10). Differences in means between the
984 two sand types are identified by t-tests with Bonferroni correction. Means with different letters are
985 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 μM 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 sand
992 types, quartz sand and mixed sand (means \pm sd; n = 5). The plants received 100 μM P (P+) or no P (0P)
993 in quartz sand. Capital letters denote differences among plant species within a P treatment, and
994 lowercase letters denote differences between the root halves for a specific element

995

996 **Table 4** Contents of non-essential elements in shoots of six species cultivated under addition of 100 μM
997 P (P+) or no P (0P) (means \pm sd; n = 5). Capital letters denote differences among the species within a P
998 treatment. Differences in element contents between species within a P treatment were identified by
999 ANOVA followed by Tukey's HSD post-hoc test. Differences in element contents in a species between
1000 P treatments were identified by t-tests with Bonferroni correction

1001

1002 **Table 5** Contents of light rare earth elements (LREE), heavy rare earth elements (HREE) in roots of six
1003 species cultivated under split-root conditions on two sand types, quartz sand and mixed sand,
1004 respectively (means \pm sd; n = 5). The plants received 100 μM P (P+) or no P (0P) in quartz sand. Capital
1005 letters denote differences among the plant species within a P-treatment, and lowercase letters denote
1006 differences between the root halves

1007

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 μ M 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
1012 mass ratios between different P-treatments are indicated by asterisks (**p < 0.01; *p < 0.05; (*)p
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 μ M 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

1027 **Fig. 3** Concentrations of trace elements in shoots (left) and roots (right) of split-root plants treated
1028 without phosphorus (low P) or with 100 μ M P at the root half growing in quartz sand. LREE = sum of
1029 La – Eu, HREE = sum of Gd – Lu plus Y (means \pm sd, n = 5). Differences between the P treatments
1030 were identified by t-tests with Bonferroni correction. In shoots, asterisks indicate significant differences
1031 between P treatments and means with the same capital letters were not significantly different (identified
1032 by ANOVA and Tukey's HSD post-hoc test) among plant species within a P-treatment at $\alpha = 5\%$. Capital
1033 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

1037 **Fig. 4** Substrate-normalized REE patterns (calculated by dividing the shoot concentrations by the
1038 average total element concentrations of the sand substrates) in A) *Triticum aestivum*, B) *Brassica napus*,
1039 C) *Pisum sativum*, D) *Cicer arietinum*, E) *Lupinus albus* and F) *L. cosentinii* treated with 100 μM P
1040 (P+) or no P (0P)

1041

1042 **Fig. 5** Stability constants ($T = 25^\circ\text{C}$ or 20°C) for REE complexation with organic acid anions. Data
1043 from Martell et al. (2004)

1044