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

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Abstract

Background and Aims A split-root approach was used to explore how phosphorus (P) nutrition influences accumulation of rare earth elements (REE) in plant species with different P-acquisition strategies beyond the commonly explored REE-phosphate precipitation.

Methods Six species (*Triticum aestivum*, *Brassica napus*, *Pisum sativum*, *Cicer arietinum*, *Lupinus albus*, and *Lupinus cosentinii*) were cultivated with a split-root system on two sand types. Phosphorus availability was controlled on one root side by watering the plants with different P-containing solutions (100 µM P, 0 µM P). Carboxylate release and changes in pH were measured on both sides. Concentrations of nutrients, cadmium (Cd), aluminium (Al), light REE (LREE: La–Eu), and heavy REE (HREE: Gd–Lu, including Y) in roots and shoots were analyzed by ICP-MS.

Results *Triticum aestivum*, *B. napus* and *C. arietinum* did not respond to a low P supply with elevated carboxylate release. These species accumulated more REE when the P supply was low and higher REE concentrations were proportional to declining plant growth. However, *P. sativum*, *L. albus* and *L. cosentinii*accumulated less REE when P-supply was low. Plants that strongly acidified the rhizosphere and released low quantities of dicarboxylates accumulated more REE (with higher LREE/HREE ratios) than species that released tricarboxylates.

Conclusion Our findings suggest that REE accumulation strongly depended on rhizosphere acidification, in concert with the amount and composition of carboxylates determining the exclusion of REE-carboxylate complexes. Leaf REE signatures may be a promising indicator as a screen tool for carboxylate-based processes in the rhizosphere using an ionomic approach.

Introduction

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 2022). Phosphorus-acquisition strategies, including carboxylate-mediated P-mining strategies, are particularly relevant in nutrient-impoverished environments; however, their relevance in plant nutrition is not restricted to environments where the availability of nutrients is very low. Even when the elements are present, their availability is often limited by the low solubility of the corresponding element-bearing minerals and interactions with inorganic and organic soil particles over a wide range of physiologically-relevant soil conditions. Plants adapted to conditions of heterogeneously distributed and sparsely available soil resources and evolved strategies to influence properties of soil surrounding their roots (rhizosphere) to create an environment more conducive for nutrient acquisition (rhizosheath). In addition to mutualistic interactions with bacteria and fungi, and alteration of soil physical properties and development of functional morphological traits (Honvault et al. 2021a, 2021b), the most profoundly studied traits involved in direct root–soil interaction include acidification of the rhizosheath by the release of protons and element-chelating carbon compounds such as carboxylates (Lambers et al. 2015; Honvault et al. 2021b; Lambers 2022). Rhizosphere acidification in the presence of element-chelating carboxylates increases the solubility and availability of many essential or beneficial elements, including P, Fe, Mn, Cu, Zn and even Si, by dissolution, complexation and ligand-exchange reactions (de Tombeur et al. 2021; Lambers 2022). The ability to mobilize Pi and micronutrients in the rhizosphere varies considerably among plant species, functional plant groups (Neumann et al. 2000; Lambers et al. 2013; Lambers et al. 2015) or even genotypes of specific species (Krasilnikoff et al. 2003). Plant species adapted to P-impoverished or P-sorbing soils, of which Proteaceae and a couple of grain legumes such as *Lupinus albus* and *L.*

cosentinii have been most profoundly studied, can respond to P deficiency by increased release of citrate and malate (Pearse et al. 2006) as a consequence of metabolic shifts in carbohydrate allocation from shoot to roots in concert with increased biosynthesis of malate and citrate and decreased citrate turnover in the tricarboxylic acid cycle (Neumann and Römheld 2000). In contrast, non-mycorrhizal phosphophilic species in the Brassicaceae, Chenopodiaceae, Urticaceae and some agricultural-relevant cereals such as *Triticum aestivum*, *Brassica napus*, *Cicer arietinum* and *Pisum sativum* cannot respond to P-deficiency with significantly elevated carboxylate release (Pearse et al. 2006; Lambers 2022).

Although carboxylate-based P-acquisition strategies are regulated by cellular nutrient supply levels 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, As, Pb, Ge and rare earth elements (REEs) (Wenzel 2009, Wiche et. 2016a, 2016b). In this respect, REEs are particularly interesting to study, because they i) are present in almost all soils at concentrations similar to essential plant nutrients (Reimann et al. 2003; Wiche et al. 2017a), ii) share chemical similarities to essential nutrients, mainly Ca (Tyler 2004; Brioschi et al. 2013), and iii) strongly interact with nutrient-bearing soil minerals (phosphates, Fe-oxyhydroxides), but are neither essential to plants nor strongly toxic (Tyler 2004; Davranche et al. 2017). The REEs comprise a group of 16 elements from the lanthanide series, including lanthanum, yttrium (Y) and scandium (Sc) that are widespread in the earth's crust with concentrations that vary from 66 µg g^{-1} (Ce), 30 µg g^{-1} (La) and 28 µg g^{-1} (Nd) to 0.3 µg 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+}; however, under most pedologically-relevant conditions, REEs form trivalent cations (Wyttenbach et al. 1998), which strongly interact with phosphate and other negatively charged soil constituents (Diatloff et al. 1993; Cao et al. 2001; Li et al., 2014). In particular, they can form stable complexes with dissolved organic compounds (Pourret et al. 2007; Wiche et al. 2017b), and the stability depends on the nature of the ligand and the REE involved.

There are slight differences in ionic radii from light REEs (LREE: La to Eu) to heavy REEs (HREE: Gd to Lu, including Y), leading to differences in their sorption and complexation behaviour in soil and their availability in the rhizosphere (Khan et al. 2016; Schwabe et al. 2021; Monei et al. 2022). For REEs in the soil solution, it is generally assumed that uptake of REE^{3+}-ions is mediated mainly by Ca^{2+}-, Na^{+}- and K^{+}-channels (Han et al. 2005; Brioschi et al. 2013), while REE-carboxylate complexes are excluded during uptake, relative to free ionic forms (Han et al. 2005; Wiche et al. 2017b). After root sorption, due to the element's higher reactivity, the biogeochemical behavior of REEs in the soil-plant system is not simply analogous to Ca^{2+}, but may resemble that of other trivalent metals, particularly Al^{3+} (Ma and Hiradate 2000). Thus root–shoot transport of REE depends on their mobility within the plant (Kovarikova et al. 2019) which is most probably through intracellular complexation with carboxylates (Ma and Hiradate 2000). Based on the above, it seems reasonable that plant species that deploy a carboxylate-based nutrient-acquisition strategy exhibit differences in REE sorption. The P status of the plants and the quantity and composition of the compounds released should influence the processes during REE mobilization and uptake.

In the present study, we conducted a split-root experiment where we cultivated six plant species (*Triticum aestivum*, *Brassica napus*, *Pisum sativum*, *Cicer arietinum*, *Lupinus albus* and *Lupinus cosentinii*) with different P-acquisition strategies and the ability to respond to P-deficient conditions by increased carboxylate release in sand. A split-root approach was used to exclude the direct effects of P-addition on REE availability, i.e. by precipitation as REE-

phosphates (Fehlauer et al. 2022; Liu et al. 2022). Thus, one root half received all essential plant nutrients except phosphate, which was supplied to one root half only. Root carboxylate release and shoot element concentrations were measured, to explore the relation between P-nutrition and plant REE accumulation. If we could show such a relation, this would offer the possibility to use shoot REE signatures as an indicator for the involvement of carboxylates during nutrient acquisition.

Methods

2.1 Substrates for plant cultivation

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 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 second sand type was added to increase the amount of potentially plant-available elements to one half 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} calcium lactate-extractable P (van Laak et al. 2018), whereas the mixed sand had a pH of 5.9 and 2.1 ± 0.3 mg kg^{-1} P. In both sand types, the total element concentrations were similar (Table 1); however, the sand types differed regarding the distribution of elements in potentially plant-available element fractions indicated by a sequential extraction analysis considering the distribution of elements in five operationally-defined soil fractions according to Wiche et al. (2017a) (Table 1). In these fractions, the mixed sand was characterized by higher concentrations of P, Mg, Ca, Mn and Fe (Table 1). Furthermore, the quartz sand showed higher concentrations of mobile/exchangeable and acid-soluble Al and higher concentrations of mobile/exchangeable LREE. Thus, in quartz sand, these elements are more easily accessible by roots than in mixed sand. However, Al and both LREE and HREE were generally more concentrated in the mixed sand, especially in the more stable fractions 4 and 5, which were also the significant element-bearing fractions of Cd (Table 1). The LREE / HREE ratios in both sand types were > 1 (Table 1). In particular, quartz sand exhibited a 12% higher LREE / HREE ratio in Fractions 1 (mobile/exchangeable) and a 15% and 33% higher LREE/HREE ratio in Fractions 4 and 5, respectively, where the elements are predominantly bound to amorphous and crystalline structures of oxides and oxide-hydroxides (Table 1). In Fractions 2 and 3, however, the LREE/HREE ratios were similar between the two substrates.

Table 1
Total element concentrations and distribution of elements in exchangeable (F1), acid-soluble (F2), oxidizable (F3) and moderately-reducible (F4) substrate constituents according to Wiche et al. (2017) determined by a sequential extraction method (mean ± sd; n = 10). Differences in means are identified by t-tests with Bonferroni correction. Means with different letters are statistically significantly different at α = 5%

Substrate	Fraction	P	K	Mg	Ca	Mn	Fe	Al	Cd	LREE	HREE
Quartz sand	Total	358 ± 50a	55695 ± 26334	4143 ± 2016	1792 ± 341	466 ± 245	1773 ± 635	5279 ± 1134	1.44 ± 0.56	12.3 ± 3.4	3.6 ± 0.9
	1	< 0.5	159 ± 15	3.9 ± 0.6b	149 ± 13b	0.4 ± 0.1b	0.8 ± 0.2	2.1 ± 0.6a	< 0.05	0.40 ± 0.03a	0.10 ± 0.01
	2	< 0.5	< 2.5	2.1 ± 0.6b	165 ± 62b	0.14 ± 0.12b	6.5 ± 1.3b	18 ± 2a	< 0.05	0.15 ± 0.03b	0.05 ± 0.01b
	3	123 ± 10b	< 2.5	2.9 ± 0.4b	128 ± 8	0.19 ± 0.10b	7.9 ± 2.0b	4.9 ± 0.6b	< 0.05	0.10 ± 0.04b	0.21 ± 0.09b
	4	26.2 ± 2.9	< 2.5	5.9 ± 0.5b	63 ± 30	0.78 ± 0.09b	89 ± 14	37.8 ± 2.6a	0.09 ± 0.01b	0.60 ± 0.06b	0.17 ± 0.03b
	5	< 0.5b	< 2.5	9.3 ± 0.9b	50 ± 14b	0.64 ± 0.14b	71 ± 31b	73.7 ± 7.2b	0.09 ± 0.01b	0.81 ± 0.13b	0.14 ± 0.03b
Mixed sand	Total	281 ± 36b	53225 ± 32089	3420 ± 2125	2015 ± 578	334 ± 125	2174 ± 748	5617 ± 1990	1.38 ± 0.34	13.8 ± 1.9	5.0 ± 1.1
	1	< 0.5	164 ± 10	10.7 ± 2.3a	339 ± 83a	2.2 ± 0.5a	1.0 ± 0.4	1.4 ± 0.2b	< 0.05	0.33 ± 0.04b	0.09 ± 0.01
	2	< 0.5	< 2.5	4.6 ± 2.5a	367 ± 266a	0.71 ± 0.42a	10.3 ± 2.4a	13 ± 3b	< 0.05	0.19 ± 0.03a	0.06 ± 0.01a
	3	165 ± 16a	< 2.5	5.1 ± 1.0a	125 ± 35	1.87 ± 1.12a	12.3 ± 2.7a	7.6 ± 1.1a	< 0.05	0.18 ± 0.05a	0.41 ± 0.17a
	4	23.8 ± 2.6	< 2.5	6.6 ± 0.4a	55 ± 9	2.17 ± 0.86a	95 ± 19	32.9 ± 3.1b	0.11 ± 0.01a	0.76 ± 0.08a	0.25 ± 0.04a
	5	8.0 ± 1.7a	< 2.5	22.5 ± 3.3a	68 ± 12a	4.44 ± 1.69a	518 ± 149a	98.7 ± 14.2a	0.12 ± 0.01a	1.03 ± 0.19a	0.24 ± 0.06a

2.2 Plant growth

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

Plant individuals with similarly developed root systems were transferred from hydroponic culture into the previously prepared pots filled with sand. Each experimental unit consisted of one plant with a split root system where one part of the root system was placed in a pot with quartz sand and the other part into a pot with mixed sand. The pots were connected with clamps, and the seedlings were stabilized with a stick to support the shoot growing between the two pots. In total, from each plant species, 10 experimental units were prepared. The plants were grown in a growth chamber at 22°C and 65% humidity, 600 µmol m^{-2} s^{-1} photosynthetically active radiation and watered with a 1/20 strength Hoagland solution containing all essential mineral nutrients, except P. After one week of growth and allowing the plants to extend their roots deeper in the sand substrates, the experimental units were watered with two different nutrient solutions containing either all essential plant nutrients according to a 1/10 strength Hoagland solution except P (P0), or all mineral elements contained in the previous solution with the addition of 100 µM P (P+). Half of the experimental units were watered with P0 solutions at both root sides (50 mL in each pot), whereas the other half received P + solutions at the root side growing in quartz sand (50 mL) and P0 solutions at the root side growing in mixed sand (50 mL). The addition of treatment solutions was continued every second day over a period of five weeks. Each P treatment was replicated fivefold for each plant species, and the different species and treatments were spatially distributed in a fully randomized design.

3.4 Rhizosphere properties and exudate collection

After five weeks, the plants were removed from the sand and carefully shaken to remove loose sand particles. Sand adhering to the root surface was collected by washing the roots with 20 mL of deionized water until 1 g of rhizosheath was obtained. The sand was left in the washing solution for 1 h until the pH was measured using a pH electrode. If necessary, the root was washed a second time without collecting the solution or sand material to remove the remaining sand entirely. The plants were transferred with their individual root systems into a 200 mL sterile Erlenmeyer flasks filled with 100 mL of a 2.5 µM $CaCl_2$ solution. This allowed the collection of root exudates depending on plant species and P-treatment for each root system separately. The plants in the collection solutions were placed back into the growth chamber and allowed to release root exudates over a time period of 3 h. Immediately after the collection, the resulting solutions were analyzed using ion chromatography. After that, the plants were separated into roots and shoots. Shoots were washed for 1 min with deionized water. The split roots were separately washed for 5 min with ice-cold $CaCl_2$ solution (5 mM) and 1 min with deionized water to remove adsorbed ions from charged root cell structures (Han et al. 2005). Finally, the shoots and roots were dried at 60°C

for 48 h, weighed and stored in centrifuge tubes until being analyzed by inductively coupled plasma mass spectrometry (ICP-MS).

3.5 Determination of carboxylates and element concentrations

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. (2002). Concentrations of P, Fe, Mn, Ca, Mg, and REEs (Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb 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. Possible interferences were monitored and corrected if necessary (Pourret et al. 2022).

Concentrations of acetate, fumarate, glutarate, malate and citrate in the collection solutions were determined by ion chromatography equipped with conductivity detection (ICS-5000, 4 mm system, Thermo Scientific, Dreieich, Germany). Inorganic and organic acid anions were separated at 30°C on 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}. The measuring program started with an eight-minute isocratic phase and a sodium hydroxide concentration of 1 mM, followed by the gradient analysis with a continuously increasing sodium hydroxide concentration of up to 40 mM for 35 min. Finally, the column was flushed for three minutes with 50 mM NaOH and equilibrated for 10 min with 1 mM NaOH.

3.4 Data processing and statistical analysis

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

Results

2.1 Plant growth and biomass responding to P-supply

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).

Considering the shoot biomass of plants without adding P, shoot biomass decreased in the order *B. napus* > *L. albus* > *P. sativum* > *T. aestivum*. The addition of P did not significantly ($p > 0.05$) affect the shoot mass of *P. sativum*, *C. arietinum*, and *L. cosentinii,* and there were no differences among *P. sativum*, *L. cosentinii* and *C. arietinum* (Fig. 1B). Here, the plant treated with 100 µM P developed only 15%, 28%, and 15% more shoot biomass than the reference plants (0P). However, *L. albus*, *B. napus* and *T. aestivum* strongly responded ($p < 0.01$) to elevated P-supply with 39%, 52%, and 88% greater shoot biomass, leading to a shift in aboveground biomass pattern of the species of *B. napus* > *L. albus* > *P. sativum* = *C. arietinum* = *L. cosentinii* = *T. aestivum* (Fig. 1B).

Considering the whole root system, including both root parts growing in quartz sand and mixed sand, the addition of P did not significantly affect root biomass within a plant species, but tended to increase ($p = 0.14$) total root mass in *L. cosentinii* by 124% compared with P-deficient plants (Fig 1C). There were no differences between plant species when the plants were externally supplied with P (Fig. 1C). However, in P0-treated plants, *L. albus* and *C. arietinum* showed the greatest root mass, and the lowest root mass was found for *L. cosentinii* (Fig 1C).

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

2.2 Shoot nutrient accumulation

Shoot [P] of plants watered with 100 µM P ranged between 1.21 mg g^{-1} (*B. napus*) to 2.46 mg g^{-1} (*T. aestivum*) (Table 2). *Triticum aestivum* and *L. cosentinii* showed substantially higher [P] in shoots than all other investigated species did. Shoot [P] of *T. aestivum*, *B. napus*, *P. sativum*, *L. albus* and *L. cosentinii* responded to a reduction in P supply by a 57%, 19%, 13%, 12% and 20% decrease of shoot [P], respectively, compared with plants treated with high P (100 µM P). Shoot [P] in *C. arietinum* was almost unchanged; however, under conditions of low P supply, *C. arietinum*, *L. albus* and *L. cosentinii* still displayed the highest shoot [P] compared with *B. napus* and *T. aestivum*.

The concentrations of K and Mg varied substantially among the tested species and ranged between 24.5 and 54.2 mg g^{-1} K and 2.7 and 6.7 mg g^{-1} Mg but did not respond to changes in P supply. As an exception, however, P supply increased shoot [K] in *B. napus* by 30%. Concerning the measured micronutrients, *L. albus* exhibited the highest [Mn] and [Fe], irrespective of P-supply. Additionally, *L. albus*, *L. cosentinii*, *B. napus* and *T. aestivum* showed higher shoot [Zn] than *C. arietinum*, the species with the lowest shoot [Mn]. The addition of low P doses tended to decrease shoot [Mn] in *T. aestivum*, *B. napus*, and *P. sativum* and significantly decreased [Fe] and [Zn] 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).

Table 2 Concentration of nutrients in shoots of six plant species cultivated under split-root conditions (means ± sd; n = 5) and addition of 100 µM P (P+) and no P in the treatment solution (0P). Capital letters denote differences among the plant species within a P-treatment

Species	P-treatment	P	K	Mg	Mn	Fe	Zn
		mg g^{-1}		µg g^{-1}			
T. aestivum	P0	1.06 ± 0.05B	49.8 ± 6.0A	2.9 ± 0.4B	160 ± 35B	122 ± 42A	46 ± 10A
	P+	2.46 ± 1.03A	51.5 ± 9.7A	2.7 ± 0.2B	206 ± 29B	99 ± 22AB	36 ± 3A
	p-value	0.02	0.75	0.34	0.06	0.32	0.10
B. napus	P0	0.98 ± 0.06B	24.5 ± 0.95B	6.2 ± 0.5A	97 ± 17C	39 ± 3B	34 ± 5A
	P+	1.21 ± 0.12B	30.2 ± 4.3B	5.9 ± 0.7A	114 ± 13BC	45 ± 8B	35 ± 7A
	p-value	0.05	0.02	0.59	0.22	0.14	0.81
P. sativum	P0	1.14 ± 0.09AB	33.0 ± 3.3B	5.4 ± 1.0A	88 ± 18BC	67 ± 10BC	27 ± 9B
	P+	1.31 ± 0.02B	37.6 ± 10.3AB	6.6 ± 2.0A	100 ± 36BC	97 ± 17AB	40 ± 7A
	p-value	0.11	0.36	0.24	0.50	< 0.01	0.09
C. arietinum	P0	1.23 ± 0.17A	28.2 ± 4.0B	3.7 ± 0.5B	63 ± 18C	84 ± 31BC	20 ± 2B
	P+	1.30 ± 0.11B	24.3 ± 1.8C	3.5 ± 0.6B	53 ± 21C	97 ± 43AB	18 ± 3B
	p-value	0.56	0.10	0.59	0.42	0.59	0.13
L. albus	P0	1.25 ± 0.15A	29.3 ± 4.3B	2.5 ± 0.3B	452 ± 96A	160 ± 71A	40 ± 8A
	P+	1.42 ± 0.071B	29.9 ± 4.9B	2.6 ± 0.6B	413 ± 51A	107 ± 30A	37 ± 6A
	p-value	0.05	0.82	0.78	0.25	0.15	0.40
L. cosentinii	P0	1.39 ± 0.17A	46.9 ± 11.3A	6.7 ± 2.2A	121 ± 58BC	88 ± 18BC	52 ± 9A
	P+	1.73 ± 0.16A	54.2 ± 19.6A	6.6 ± 2.0A	96 ± 38BC	87 ± 20AB	46 ± 6A
	p-value	0.03	0.55	0.94	0.56	0.91	0.33
Species	P0	<0.001	<0.001	<0.001	< 0.001	< 0.01	< 0.01
	P+	<0.01	<0.01	<0.001	<0.001	< 0.01	< 0.01

2.3 Root nutrient accumulation

Root [P], [Mn] and [Fe] varied substantially among plant species, P supply and the root part considered (roots growing in quartz sand and mixed sand, respectively) (Table 3). Considering the root part growing in quartz sand, where P-supply was controlled, the addition of low P solutions (0P) decreased root [P] in this root part of all plant species by 10–25% compared with plants treated with 100 µM P (P+). This effect was strongest in *T. aestivum*, *B. napus*, *P. sativum* and *C. arietinum* ($p < 0.05$) and somewhat weaker in *L. albus* and *L. cosentinii* ($p > 0.05$). Considering both P treatments, *L albus* showed the lowest root [P], and *T. aestivum*, *B. napus*, *P. sativum* and *L. cosentinii* exhibited the highest root [P], especially when the plants received high P-doses (P+).

Table 3 Nutrient concentrations in roots of six plant species cultivated under split-root conditions on two sand types, quartz sand and mixed sand (means ± sd; n = 5). The plants received 100 µM P (P+) or no P (0P) in quartz sand. Capital letters denote differences among plant species within a P treatment, and lowercase letters denote differences between the root halves

		Root part with P supply (quartz sand)			Root part without P supply (mixed sand)		
Species	treatment	P	Mn	Fe	P	Mn	Fe
		mg g^{-1}	µg g^{-1}		mg g^{-1}	µg g^{-1}	
T. aestivum	P0	1.34 ± 0.25aAB	103 ± 28bA	8345 ± 2889aA	0.93 ± 0.13bC	175 ± 49aA	1784 ± 440bA
	P100	1.78 ± 0.23aA	131 ± 27bAB	6270 ± 2032aA	1.15 ± 0.09bC	252 ± 45aA	2614 ± 585bA
	p-value	0.02	0.14	0.13	0.01	0.06	0.04
B. napus	P0	1.54 ± 0.19A	148 ± 83A	1243 ± 697B	1.53 ± 0.10A	97 ± 23B	391 ± 97C
	P100	1.79 ± 0.11aA	86 ± 40AB	476 ± 132C	1.56 ± 0.06bA	67 ± 21C	316 ± 48D
	p-value	0.03	0.23	0.04	0.65	0.10	0.31
P. sativum	P0	1.52 ± 0.13A	152 ± 109AB	1692 ± 792B	1.39 ± 0.08A	165 ± 71AB	1213 ± 434AB
	P100	1.68 ± 0.04aA	64 ± 35B	1489 ± 34aB	1.35 ± 0.07bB	90 ± 26BC	1100 ± 89bB
	p-value	0.06	0.24	0.68	0.44	0.08	0.63
C. arietinum	P0	1.32 ± 0.09aAB	129 ± 43bA	1515 ± 260aB	1.15 ± 0.06bB	205 ± 30aA	1014 ± 82bB
	P100	1.85 ± 0.30aA	213 ± 126A	1909 ± 799aB	1.21 ± 0.07bBC	133 ± 17B	758 ± 171bC
	p-value	<0.01	0.20	0.38	0.15	<0.01	0.02
L. albus	P0	1.10 ± 0.26B	47 ± 8B	1325 ± 250aB	1.08 ± 0.04B	43 ± 8C	632 ± 79bB
	P100	1.35 ± 0.08B	44 ± 14B	919 ± 145aB	1.31 ± 0.17BC	36 ± 5C	790 ± 109bC
	p-value	0.09	0.72	0.01	0.03	0.18	0.25
L. cosentinii	P0	1.38 ± 0.17A	134 ± 23A	2691 ± 2035B	1.20 ± 0.04B	123 ± 77AB	1075 ± 537AB
	P100	1.63 ± 0.45AB	81 ± 53AB	2610 ± 1567B	1.30 ± 0.05BC	68 ± 21C	1084 ± 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

When P supply was high, *T. aestivum*, *B. napus*, *C. arietinum* and *L. cosentinii* showed higher root [Mn] than *L. albus*, and the reduction of P-supply did not significantly influence [Mn] of roots in quartz sand. However, P-deficient *L. cosentinii* had a 65% higher [Mn] than P-supplied plants (p = 0.12). *Triticum aestivum* exhibited the highest [Fe]

compared with all other species, irrespective of P-treatment, and *B. napus* the lowest. Reduction of P supply increased root [Fe] in *B. napus* and *L. albus* by 161% and 44%, respectively.

In the corresponding mixed sand root part of P-supplied plants, root [P] was the highest in *B. napus* and *P. sativum* and the lowest in *T. aestivum*. When P supply was reduced at the root side in quartz sand, root [P] also declined significantly in the mixed sand root part of *T. aestivum* (19%) and *L. albus* (18%) but was unchanged in the other species. Considering both P treatments, roots in mixed sand of *T. aestivum* and *C. arietinum* showed consistently lower [P] than roots growing in quartz sand, while in *B. napus* and *P. sativum* root [P] only differed when P was added to the root half in quartz sand (higher P in quartz sand than in mixed sand). *Lupinus albus* and *L. cosentinii* did not show any differences in root [P] between the roots when P was added, nor in situations of P deficiency. Concerning the micronutrients, roots in mixed sand of *T. aestivum* had the highest [Fe] and [Mn] and *L. albus* showed by far the lowest concentrations. The [Mn] and [Fe] of the root half in mixed sand generally responded more strongly to the P-treatment than the root half growing in quartz sand. Specifically, *T. aestivum* showed the highest root [Mn] when supplied with P; however, the reduction in P supply reduced root [Mn] and [Fe] in quartz sand by 31% and 32%, whereas root [Mn] increased in *B. napus* (45%), *P. sativum* (83%), *C. arietinum* (54%), *L. albus* (20%) and *L. cosentinii* (81%). Moreover, root [Fe] increased by 34% in *C. arietinum* but was unchanged in the other species.

3.4 Carboxylate release in response to P supply

Considering the quantity of carboxylates released by both root parts per root half and unit of time, *B. napus* released by far the greatest amounts, irrespective of P-treatment (Fig. 2A). In *B. napus*, *T. aestivum* and *P. sativum,* the major portion (more than 98%) of the carboxylates released consisted of malate, and citrate was only occasionally detected. In contrast, *C. arietinum*, *L. albus* and *L. cosentinii* released both malate and citrate (Fig. 2A). Carboxylate release was not affected by P supply in *T. aestivum* and *C. arietinum*. *Brassica napus* and *P. sativum* responded to a reduction in P supply by a decrease in carboxylate release by 20% ($p = 0.04$) and 44% ($p = 0.08$), respectively. In contrast, in *L. albus* and *L. cosentinii*, the reduction of P supply significantly increased total carboxylate release by 159% ($p<0.01$) and 115% ($p = 0.03$), respectively, showing an increase of both malate and citrate, but especially of citrate (Fig. 2A).

Roots growing in mixed sand released greater amounts of carboxylates per unit time in all tested plant species, except *B. napus*, which tended to release greater amounts of malate in quartz sand, but only when this root part was supplied with P (Fig. 2B). Also, in the other species, there were significant differences in the response of the different root halves to P supply. *Triticum aestivum* showed no response in any of the root halves (Fig. 2B). *Brassica napus*, *P. sativum* and *C. arietinum* predominantly responded in the root half in quartz sand, where P was added with the watering solution and showed a significant reduction in carboxylate release (24%, 65% and 75%) at low P supply. In comparison, in the root half in mixed sand, carboxylate release in *P. sativum* and *C. arietinum* was unchanged or increased in *B. napus* by 80% when P supply in quartz sand was reduced. Also, *L. albus* and *L. cosentinii* did not respond in the root half of the P supply but showed an increase of carboxylate release from the root half in mixed sand only. Here, the exudation of malate increased by 121% and 320%, respectively, and citrate release increased by 192% and 870%, respectively, when P-supply was low at the root half in quartz sand (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, similarly to the carboxylate release per root (Fig. 2B), showing decreasing carboxylate release at the root half in quartz sand (*C. arietinum*: 90% decrease) or increasing exudation at the root half in mixed sand (*L. albus*: 105% increase) (Fig. 2C).

3.5 Rhizosheath acidification in response to P supply

In the rhizosheath of all plant species and treatments, the pH was significantly higher than that of the unplanted control soil (Fig. 2D). The pH in the mixed sand rhizosphere was consistently higher (on average 0.3 units considering all species) than that in quartz sand as a consequence of the initial pH of the substrates used (Table 1); however, the pH of the substrates was altered depending on plant species root half and P supply (Fig. 2D). Considering data from both root halves, the rhizosphere pH of P-supplied plants of *B. napus*, *L. albus* and *L. cosentinii* (pH 7.1 ± 0.2) was on average 0.5 units higher than that of *C. arietinum*, *P. sativum* and *T. aestivum* (pH 6.6 ± 0.1). When the P supply was low, the pH in the soil of *B. napus* was still highest but lowest in *C. arietinum* and *T. aestivum* (pH 6.4) and the differences initially observed between *P. sativum*, *L. cosentinii,* and *L. albus* disappeared which was predominantly driven by strong acidification of the rhizosphere of *L. cosentinii* and *L. albus,* especially at the root half in mixed sand, at a low P supply. In contrast, in *P. sativum*, the pH was not strongly affected by P supply and was comparatively low, irrespective of P-treatment.

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* = *C. arietinum*. When P was lacking, the rhizosphere pH of *B. napus*, *C. arietinum* and *T. aestivum* was 0.3 units lower ($p < 0.05$) but unchanged (around 6.7 ± 0.2) for *L. albus*, *L. cosentinii* and *P. sativum*.

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 root rhizosheath of *L. albus* and *B. napus* by 0.2 units and strongly reduced the pH in the rhizosheath of *L. cosentinii*, by 0.6 units. In contrast, there were no pH changes in the rhizosheath of *C. arietinum* and *T. aestivum*.

3.5 Shoot element accumulation responding to P supply

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

Considering the shoot element contents (calculated as shoot biomass x concentration) (Table 4), *B. napus* showed the highest Cd, Al and REE contents, mainly when the plans were supplied with P and shoot content was lowest in *T. aestivum*. The low P supply did not significantly affect the shoot REE content of *T. aestivum*, *L. cosentinii*, *C. arietinum* and *B. napus*. However, in the latter species, the contents of both LREE and HREE tended to be by 21–34% lower. Also, *in L. albus* and *P. sativum,* LREE and HREE contents were 40–46% (*P. sativum*) and 58–60% (*L. albus*) lower at low P supply. Also, in *L. albus* Al, Cd contents were 48% and 71% lower. Shoot Al contents in *B. napus* was 47%, lower and Cd content in *T. aestivum* was 43% lower when the P supply was low. In contrast, Al and Cd contents in *P. sativum*, *C. arietinum* and *L. cosentinii* were largely unaffected by P supply.

Table 4 Contents of non-essential elements in shoots of six species cultivated under addition 100 µM P (P+) or no P (0P) (means ± sd; n = 5). Capital letters denote differences among the plant species within a P-treatment

		Element contents			
Species	Treatment	Al	Cd	LREE	HREE
		µg	ng	µg	µg
T. aestivum	P0	14 ± 3B	13 ± 4B	0.11 ± 0.02C	0.03 ± 0.01C
	P100	20 ± 12B	23 ± 5BC	0.14 ± 0.08C	0.04 ± 0.02C
	p-value	0.36	0.05	0.52	0.31
B. napus	P0	68 ± 10A	526 ± 125A	3.4 ± 0.9A	0.44 ± 0.15A
	P100	128 ± 62A	451 ± 121A	4.3 ± 1.0A	0.64 ± 0.18A
	p-value	0.03	0.36	0.19	0.12
P. sativum	P0	59 ± 36AB	20 ± 7B	1.24 ± 0.33B	0.21 ± 0.04B
	P100	55 ± 32AB	24 ± 5BC	2.05 ± 0.34B	0.39 ± 0.05B
	p-value	0.35	0.41	0.02	<0.01
C. arietinum	P0	31 ± 15B	9 ± 4B	0.46 ± 0.24BC	0.10 ± 0.06BC
	P100	76 ± 59AB	10 ± 3C	0.70 ± 0.29C	0.16 ± 0.08C
	p-value	0.17	0.72	0.18	0.21
L. albus	P0	56 ± 18AB	23 ± 9B	0.77 ± 0.39B	0.18 ± 0.09B
	P100	107 ± 56A	79 ± 55B	1.82 ± 0.55B	0.45 ± 0.12B
	p-value	0.06	0.04	0.01	0.04
L. cosentinii	P0	22 ± 14B	23 ± 10B	0.26 ± 0.16C	0.05 ± 0.02C
	P100	33 ± 22AB	26 ± 9BC	0.17 ± 0.07C	0.06 ± 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

3.6 Root element contents in response to P supply

Element concentrations in roots varied substantially depending on plant species and root halves (quartz sand and mixed sand, respectively). They were additionally affected by P supply (Fig. 3). All investigated plant species showed significantly higher [LREE] and [HREE] in roots growing in quartz sand, irrespective of the P treatment. Similarly, quartz sand roots of *L. albus* and *L. cosentinii* exhibited higher [Al] and [Cd]. *Brassica napus* showed higher [Al] and *P. sativum* showed higher [Cd] in quartz sand roots than in mixed sand, but in plants exposed to low P solutions. In contrast, *T. aestivum* showed higher [Cd] in mixed sand roots, but only when P was supply wat high. Considering the different P supply, 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 compared with roots supplied with P. In *P. sativum*, P deficiency also increased [Cd] by 89% and decreased [HREE] by 38% but did not affect [Al] and [LREE]. In the corresponding mixed sand roots of *C. arietinum*, *L. albus* and *L. cosentinii*, the addition of P to the roots in quartz sand increased [Cd] in all three species, increased [LREE] and [HREE] in *C. arietinum* (80% and 59% increase) and [LREE] in *L. albus* (30% increase), whereas [HREE] was unaffected in *L. albus*. In none of the species, there were changes in [Al] in the roots growing in mixed sand, neither in the lupin species nor in *T. aestivum*, *B. napus* and *P. sativum*. However, in *B. napus* [Cd], [LREE] and [HREE] was significantly higher, by 47%, 66% and 69%, at a low P supply. In contrast, in roots of *P. sativum* grown in mixed sand, concentrations of all abovementioned elements were not altered by affected by P supply. Also, in *T. aestivum,* [Cd] was not changed, but, as an exception, [LREE] and [HREE] were 25% and 20% lower at low P supply. Considering data from both root parts and P treatments, the calculated LREE/HREE ratios of *L. albus* and *C. arietinum* were substantially higher than those in the other species (on average 4.9–5.0) and the lowest values was in *L. cosentinii* (LREE/HREE = 3.6 ± 0.7). The LREE/HREE ratios were higher in roots grown in quartz sand than those in mixed sand, except for *T. aestivum* and *P. sativum*. In *T. aestivum*, the ratios were higher in roots grown in mixed sand of P-supplied plants than in corresponding roots grown in quartz sand but without differences between the P+ and P0 treatments. In contrast, in *P. sativum*, adding P to the quartz sand decreased the ratio from 5.0 to 4.3. Similarly, in *L. albus*, P addition decreased the LREE/HREE ratios from 5.7 to 5.0 in roots grown in quartz sand and 4.6 to 4.1 roots grown in mixed sand.

Considering the root element contents (calculated as root biomass x concentration) sorbed by the different root halves, the P-supplied roots of *C. arietinum* growing in quartz sand accumulated more REEs than all other investigated species except *P. sativum,* which showed similar net root uptake of LREE and HREEs. We found the lowest REE accumulation in the roots of *T. aestivum* and *L. cosentinii*, compared with the other species, including *L. albus*. In contrast, *L. albus* showed the highest contents of Cd and Al. In the other root half growing in mixed sand, there were no differences in element contents between the species, except for *T. aestivum* and *C. arietinum,* which showed a 3-4 times higher Cd content compared with *B. napus*, *P. sativum* and *L. cosentinii*. A low P supply at the root half in quartz sand did not change the contents of Al and Cd in any of the investigated species, neither in quartz sand nor in mixed sand roots. However, in roots grown in quartz sand, LREE and HREE contents were 45% lower in *P. sativum*, while LREE and HREE contents were constant in the other species. Conversely, reducing the P supply in roots grown in mixed sand did not change LREE and HREE contents in *P. sativum* and did not affect LREE in the other species. However, in *B. napus*, the low P supply tended to increase the content of HREE by 83%, while in *L. cosentinii*, the root uptake decreased by 44%.

Table 5 Contents of light rare earth elements (LREE), heavy rare earth elements (HREE) in roots of six plant species cultivated under split-root conditions on two sand types, quartz sand and mixed sand, respectively (means ± sd; n =

5). The plants received 100 µM 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

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

3.7 Normalized REE pattern in shoots of plant species and responses to P supply

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

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

Discussion

3.1 Plant growth, root biomass and nutritional status

In this study, we tested six plant species previously characterized by Pearse et al. (2006) regarding their ability to acquire P from different P forms in soil by carboxylate release and rhizosphere acidification. In the present experiment, the cultivation of plants with split roots growing in different sand types allowed us to control the P nutrition status on one root side without influencing REE availability directly through the precipitation of Al and REE with phosphate in the presence of P. The treatment with low P supply showed less production of shoot and total plant biomass of *T. aestivum*, *B. napus*, and *L. albus,* whereas there was no effect on *C. arietinum*, *P. sativum* and *L. cosentinii* (Fig. 1). In the latter species, no response to different levels of P addition might be explained by a virtually unchanged shoot [P] following P addition. Indeed, when P was added to the root half in quartz sand, shoot [P] increased in all species, except in *P. sativum* and *C. arietinum* (Table 2). This was unexpected, given that the plants received a high supply of P (100 µM P as KH_2PO_4) in the nutrient solutions. Shoot [P] did not exceed the concentration that is adequate for crop growth of 2 mg P g^{-1} dry weight (Marschner 1995), except in *T. aestivum*, suggesting that the amount of P given to the plants was not sufficient to achieve a luxury uptake in the P-treated plants and the amount of P given may have been sorbed onto Al and Fe oxides and hydroxides under the acidic conditions of the quartz sand. After five weeks of plant growth, all plants entered the reproduction phase, so P remobilization to the seeds may have contributed to the low shoot [P] (El Mazlouzi et al. 2020). Shoot [Fe] and [Mn] were largely unchanged at the low P supply, except in *T. aestivum* and *P. sativum*, where [Mn], [Fe] and [Zn] were lower that at a high P supply (Table 2). Given that these elements were added to the nutrient solution irrespective of the P-treatment, this suggests a reduced uptake and/or translocation capacity under conditions of P deficiency (Fan et al. 2021). Root [P] was higher in all species at a high P-supply. This higher root P status was not only observed in the root half in contact with the nutrient solution, but also roots grown in mixed sand of *T. aestivum*, *C. arietinum* and *L. albus*, and to some extent in roots of *L. cosentinii*. Conversely, [P] of roots grown in mixed sand of *B. napus* and *P. sativum* were unaffected. Indeed, the [P] was highly influenced by root growth. However, all plants developed

more root biomass in the mixed sand. Hence, these findings indicate that the plants allocated a large portion of P absorbed in quartz sand to the other root half growing in mixed sand.

The increased root mass ratios of *T. aestivum*, *B. napus* and *L. albus* in P0 treatments (Fig. 1) indicate a relatively increased allocation of dry matter to roots and adjustment of root growth to a low P supply (de Bang et al. 2020). This growth adjustment is regulated by the overall nutrient status of the plants (Robinson 1996), and, therefore, might explain the high biomass allocation in *T. aestivum*, which showed the largest differences in shoot [P] resulting from differences in P supply. In contrast, *L. cosentinii*, *C. arietinum* and *P. sativum* did not respond to differences in P supply with altered root mass ratios. These species presumably relied more heavily on chemical changes in the rhizosphere than on more extensive root systems (Pearse et al. 2006). In the present experiment, the mixed sand (roots without P supply) was characterized by a higher pH, higher calcium acetate-extractable P availability (Table 1). Therefore, in the present experiment, resource allocation must be considered not only between shoot and root but also between the different root halves (Fig. 1), allowing us to explore the capacity to respond to nutrient availability by plasticity in root development. Indeed, when P supply was low in quartz sand (P0), all species (except *L. cosentinii*) developed more extensive roots in the mixed sand where the plants were exposed to conditions that allowed them to acquire more nutrients. The P0 treatment reduced the root growth of *B. napus* in quartz sand, but did not affect the root mass of other species at this root side.

When the P supply was higher at the root side of quartz sand, the root mass of *L. albus* was unaffected in mixed sand, but *B. napus* showed a lower root mass. In contrast, *L. cosentinii* and *C. arietinum* had a higher root mass in mixed sand (Fig. 1) when the plants were supplied with P to the roots in quartz sand. This suggests that under P deficiency, the phosphophile *B. napus* mainly relies on readily-available P sources and effectively adjusts its root growth to the compartment where P can be most easily acquired. In contrast, *C. arietinum*, *L. albus* and especially *L. cosentinii* appeared to follow a more conservative strategy and sustained root development in the mixed sand with higher total nutrient concentrations, increasing the chance to maintain P and micronutrient supply in nutrient-impoverished environments, where the presence of nutrients and their availability is patchy. In particular, this plasticity in root development, in concert with the species' ability to respond to P deficiency by altered carboxylate release and rhizosphere acidification (see Section 4.2), might contribute to the result that *L. cosentinii* yields are twice as high as those of *L. albus* under low-P conditions in the field (Rahman an Gladstones 1974).

3.2 Modifications of rhizosphere chemistry in response to P supply

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 P nutritional 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 influence the root environments chemically. In contrast, exudation rates per unit of time and root mass characterize the physiological response to environmental conditions. The exudation rates per unit root mass as dependent on P supply were not as pronounced as the carboxylate release per unit time, most probably due to a declining exudation rate at the reproductive stage (Mimmo et al. 2011). *Triticum aestivum*, *B. napus*, *P. sativum* and *C. arietinum* did not show differences in rhizosphere pH in response to P supply (Fig 2D);

however, the rhizosheath pH was lowest in *P. sativum* and *C. arietinum* (Fig. 2D), highlighting the capacity of these species to acidify the rhizosphere irrespective of P supply, which is consistent with the findings of Pearse et al. (2006) who demonstrated strong rhizosphere acidification for *C. arietinum* independent from P supply. The lupins (*L. albus*, and *L. cosentinii*) strongly acidified the rhizosphere when P was lacking in the nutrient solution, especially in the mixed sand, which was characterized by higher nutrient availability. It is generally assumed that the response of plants to nutrient deficiency is regulated by the overall nutrient status of the plants, as demonstrated for cluster-root formation and carboxylate release in lupins and some Proteaceae species (Shane et al. 2003a, 2003b; Wang et al. 2013). However, In *C. arietinum,* the production and release of carboxylates appears to be independent of plant P status (Wouterlood et al. 2004) and *B. napus* and *T. aestivum* typically show declining carboxylate release under P-deficient conditions (Pearse et al. 2006). Consistently, in the present study, P deficiency increased the carboxylate release of the lupins, reduced carboxylate release in *B. napus* and *P. sativum*, but did not affect the amount of carboxylates released in *C. arietinum* and *T. aestivum* (Fig. 2A, B), demonstrating some species lack the capacity to respond to P supply.

Despite the lower carboxylate release of *B. napus* at a low P-supply, *B. napus* released the greatest amounts of carboxylates, mainly malate. Dicarboxylates such as malate, succinate and fumarate are not quite as effective in mobilizing P and trace nutrients in the soil as tricarboxylates are, mainly due to the lower complex stabilities for malate compared to citrate (Jones 1998). In contrast, citrate, a tricarboxylate, forms more stable complexes with soil cations and consequently is more efficient in releasing nutrients by complexation and ligand exchange reactions as a P-mining strategy (Lambers 2022). Citrate was predominantly found in exudates of the P-efficient lupins, *L. albus* and *L. cosentinii* (Fig. 2A), attributing to these species' ability to respond to a low P supply by acquiring P and micronutrients from sparingly soluble soil phases (Lambers 2022). When P supply was low in the quartz sand substrate, all species responded with decreased amounts of carboxylate release at this root side (Fig. 2B), which was primarily due to reduced root mass as a consequence of P starvation (Fig. 1). Moreover, *B. napus*, *P. sativum* and *C. arietinum* were characterized by declining exudation rates of roots in quartz sand (Fig. 2C). In contrast, carboxylate-exudation rates were unaffected in P-starved roots of *L. albus* and *L. cosentinii* and these species showed an up-regulation of carboxylate release in roots in mixed sand (Fig. 2B, C). Moreover, roots of lupins in mixed sand showed decreasing P concentrations (Table 3) when roots in quartz sand were exposed to low P concentrations in the watering solution. This plasticity in root system development in concert with adjustment of root activity and resource allocation would be especially advantageous when different species or roots of the same species compete for sparingly available soil nutrients (Hodge et al. 1999) that, even if present, are typically heterogeneously distributed in soil (Hinsinger et al. 2009).

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

A low P supply may affect the accumulation of non-essential elements through i) altered plant growth and thus an enrichment per unit biomass, ii) altered uptake and translocation when uptake is mediated by nutrient transporters that are affected by the growth-limiting nutrient, and iii) altered solubility and chemical speciation in the rhizosphere determining the accessibility for transport mechanisms. If altered solubility is involved, when the availability is limited by mobility in soil, any increase in solubility following changes in chemical speciation will ultimately increase diffusion towards the root and the probability of the element entering the root. Conversely, when the mobility of elements is high(er), changes in the chemical speciation from the ionic form to a metal-organic complex may decrease availability through exclusion at the site of uptake (Barber and Lee 1974).

In the present experiment, all plants altered the rhizosheath pH and released carboxylates depending on species and P supply (Fig. 2). The sand substrates contained the elements in sparingly soluble forms (Table 1). In both substrates, cadmium availability was associated with amorphous and crystalline silicates (Wiche et al. 2017a), and less than 0.1% of Cd, Fe, Mn, and Al were present in mobile forms (Fraction 1). In contrast, the solubility of REE was somewhat higher, especially in the quartz sand (Table 1). Nonetheless, all species contained detectable concentrations of all elements with high variability among the species tested (Fig. 1). Aluminum and REE showed a similar behavior in the shoots, consistent with the literature (Liu et al. 2021; Fehlauer et al. 2022). In *B. napus*, high shoot and low root [Cd] can be primarily explained by the efficient influx and transport of Cd from roots to shoots (Selvam and Wong 2009).

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

However, reduced Cd contents in shoots of *T. aestivum* (Table 3) might be explained by a lower Cd acquisition from the quartz sand, where the plants tended to develop less biomass at a low P supply (Fig. 1) together with a slightly reduced carboxylate release at the root half in mixed sand (Fig. 2). Similar to *T. aestivum*, the shoot biomass of *B. napus* was lower as a consequence of P supply (Fig. 1) but without changes in Cd, Al and HREE concentrations (Fig. 3), whereas LREE concentrations were significantly higher (Fig. 3) and Al, LREE, and HREE contents were less. (Table 3). Additionally, in P-deficient plants, total carboxylate release was less (Fig 2), suggesting that the element pattern in shoots was a consequence of a less element acquisition in concert with a preferential root-shoot transfer of LREE relative to HREE and LREE accumulation in shoots. HREE form more stable complexes with low-molecular-weight organic anions, presumably citrate, during long-distance transport in the xylem (Ma and Hiradate 2000). However, based on the higher charge density, HREE are preferentially sorbed onto cell walls during radial transport and form more stable complexes with metabolites released into the rhizosphere. Given that REEs are predominantly taken up in ionic form through Ca, K, and Na channels (Han et al. 2005), carboxylates and other chelating compounds would alter the chemical speciation, and hence the uptake and accumulation of REE, including the ratio of LREE/HREE (Wiche et al. 2017b).

Element exclusion through extracellular complexation has been studied in detail for Al in Al-tolerant species (Zheng et al. 1998; Ma et al. 2001; Kochian et al. 2004) and Cd in *L. albus* (Römer et al. 2000). For a specific carboxylate (e.g., citrate), the complex stabilities decrease in the order HREE > LREE > Al > Cd (Byrne and Li 1995; Martell et al. 2004), while for a given element (e.g., La), the complex stabilities decrease in the order citrate > malate > acetate (Fig. 5).

Han et al. (2005) demonstrated that organic acids promote the uptake of La by barley, but the effect of the acid decreased in the order acetic acid > malic acid > citric acid, which can be explained mainly by decreased sorption of La onto the apoplast in the presence of the acid anion but a reduced uptake with increasing complex stability (Han

et al. 2005). In the present experiment, *B. napus* released large quantities of malate (Fig. 2), a dicarboxylate with lower complexation constants (La: log K 4.37) compared with citrate (La: log K 7.63), but the large quantities released should favour complex formation and element exclusion, which might also explain the lower total REE concentrations in *B. napus* compared with *P. sativum* which released much lower amounts of dicarboxylates but strongly acidified the rhizosphere (Fig. 2D) and mobilized the elements in plant-available (ionic) forms (Cao et al. 2001; Wiche et al. 2017b). Slight differences in the complexation behaviour between LREE and HREE might have influenced the LREE accumulation in this species at a low P supply (Figs 3; 4). Indeed, P-deficient roots exposed to quartz sand with higher mobility of REE (Table 1) showed higher concentrations of Al, LREE and HREE but unaffected net root sorption (Table 3) with lower carboxylate release (Fig. 2). Conversely, P-deficient roots in mixed sand released greater amounts of carboxylates (Fig. 2B) and showed higher concentrations (Fig. 3) and element contents (Table 3), most likely through increased element dissolution followed by decreased internal element transport. This contention is further supported by the responses in *P. sativum*, *L. albus* and *L. cosentinii*. *Pisum sativum* strongly acidified the rhizosheath in both root parts, irrespective of P supply, and released only small amounts of carboxylates, especially malate (Fig. 3). A reduction in P-supply did not change shoot and root biomass (Fig. 1). Still, it decreased the concentrations and contents (Fig. 3) of LREE, HREE and Fe, Mn, and Zn with higher LREE/HREE ratios in P-deficient plants. Conversely, in shoots of *L. albus* and *L. cosentinii*, the concentrations and contents of LREE, HREE and Cd declined (Fig. 3) at a low P-supply, which was accompanied by greater exudation of citrate and malate (Fig. 2). Although in *L. cosentinii*, this effect was somewhat less pronounced than in *L. albus*, in *L. cosentinii* P-deficient plants displayed significantly lower LREE/HREE ratios indicating a higher HREE translocation relative to LREE when P-supply was low. In contrast, P-deficient roots of *L. albus* showed higher LREE/HREE ratios, irrespective of the root half, while in *L. cosentinii*, the LREE/HREE ratios in roots were unaffected. This can be primarily explained by the strong acidification of the rhizosphere of *L. cosentinii,* shifting the carboxylic acid: carboxylate ratio towards the acid form (Pearse et al. 2006), preventing complex formation and favouring uptake of LREE in *L. albus* but not in *L. cosentinii*, where the presence of carboxylates might have increased the release and uptake of HREE from sparingly-available element forms from the HREE enriched mixed sand (Table 1).

3.4 Conclusion

We demonstrated that P-nutrition influenced the accumulation of the non-essential elements Cd, Al, and REE, beyond the commonly recognized mechanism of REE-phosphate precipitation in roots. Plants that strongly acidified the rhizosphere and released small quantities of dicarboxylates accumulated the highest concentrations of REE. Conversely, low rhizosphere acidification and large amounts of carboxylates were associated with a significantly lower accumulation of REE. Phosphophile species or plants that do not respond to P deficiency (*B. napus*, *T. aestivum*, *C. arietinum*) with increased carboxylate release accumulated REE to higher concentrations when P supply was low, which could be explained largely by reduced growth and this concentration of REE and enrichment of the elements in the plant biomass. In contrast, plants that released more tricarboxylates under conditions of P deficiency accumulated more REE when the P supply was high and carboxylate release was low. The proposed mechanism involves the mobilization of the elements in the rhizosphere through carboxylate and proton release, pH-dependent formation of REE-carboxylate complexes with complex stabilities depending on the amount and composition of carboxylates with HREE-complexes > LREE-complexes and exclusion of the complexes during uptake, radial transport and/or translocation. This suggests a functional overlap of carboxylate-based belowground traits related to P nutrition and exclusion of REE, which otherwise might become toxic in REE-enriched growth environments. The relationship between plant nutrition and REE accumulation could also explain the large variability in REE accumulation among different plant species and plant individuals growing in the same soil. The

proposed model delivers a mechanistic explanation for the REE-hyperaccumulation in Proteaceae (Van der Ent et al. 2022) and highlights the potential of leaf REE signatures to characterize plant species regarding their P-acquisition strategy through changes in rhizosphere chemistry following an ionomic approach.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Oliver Wiche, Christine Dittrich, Nthati Monei and Juliane Heim. The first draft of the manuscript was written by Oliver Wiche and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

All data obtained during the experiment are contained in the manuscript

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Figures

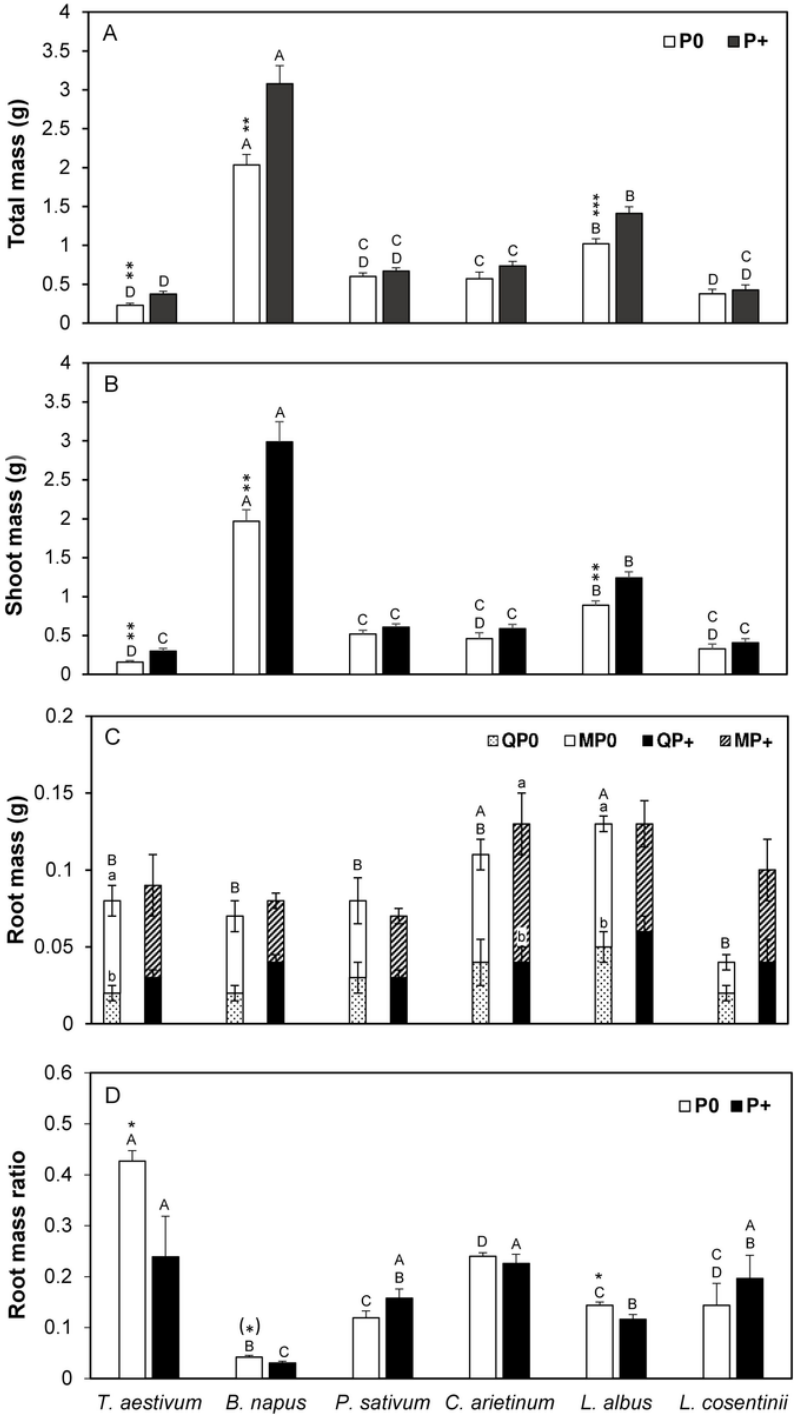

Figure 1

Shoot mass and root mass (g) of the two root halves growing in quartz sand (Q) and mixed sand (M) and root mass ratio considering both root halves depending on treatment with 100 µM P (P+) and no P (0P). The bars represent means ± se (n = 5). ANOVA identified differences among species (capital letters) following Tukey's HSD post-hoc test at α = 5%. Significant differences in shoot mass and root mass ratios between different P-treatments are indicated by asterisks (**p < 0.01; *p < 0.05; (*)p <0.1), while differences in root masses among the root halves are indicated with lowercase letters. Means with the same letters are not significantly different at α = 0.05

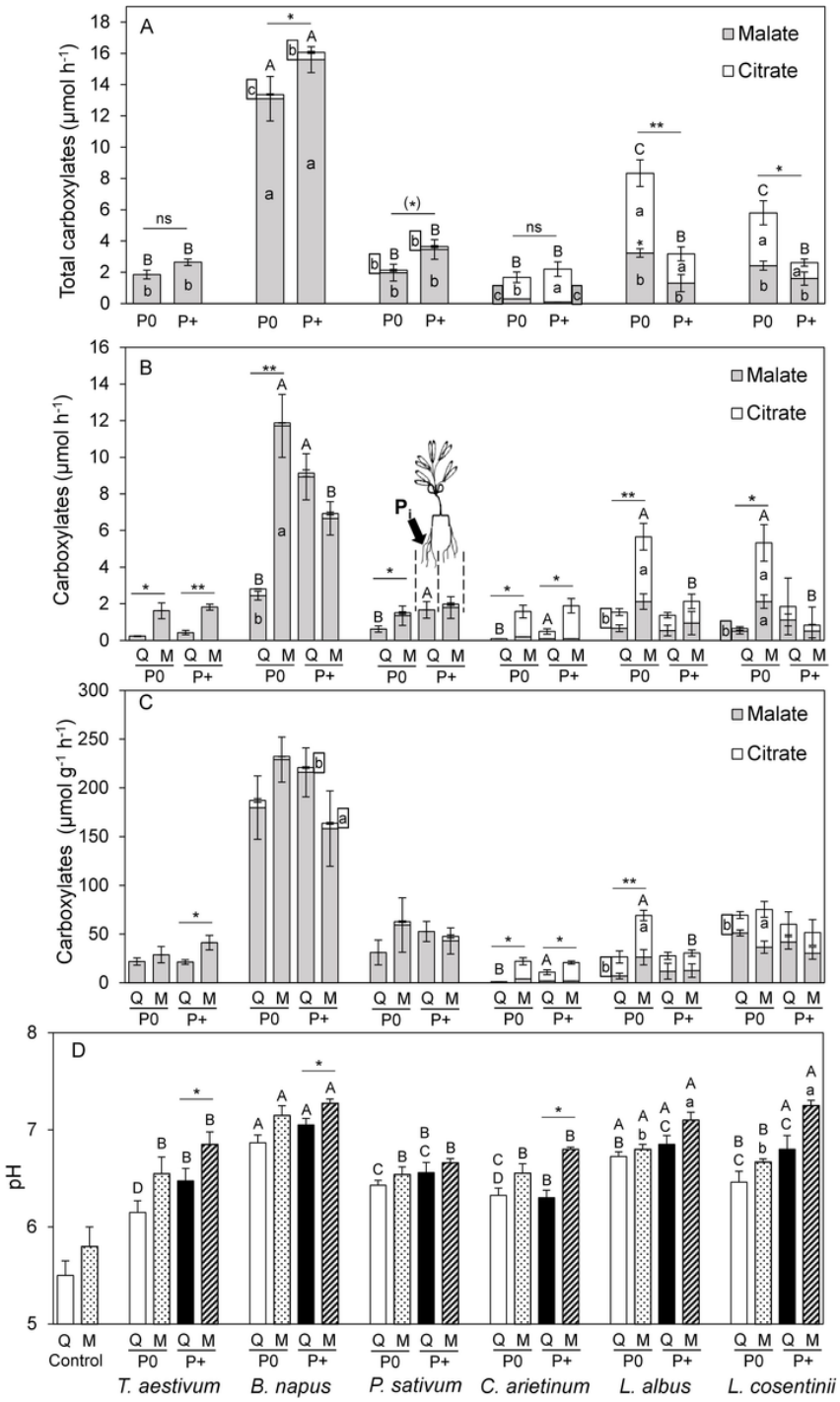

Figure 2

Total carboxylate release per plant (A), (B) carboxylate release from root halves growing in quartz sand (Q) and mixed sand (M) (µmol h^{-1}), (C) exudation rates (µmol g^{-1} h^{-1}) from the different root halves, and (D) rhizosphere pH depending on treatment of plants with 100 µM P (P+) or no P (0P) from the root half growing in quartz sand

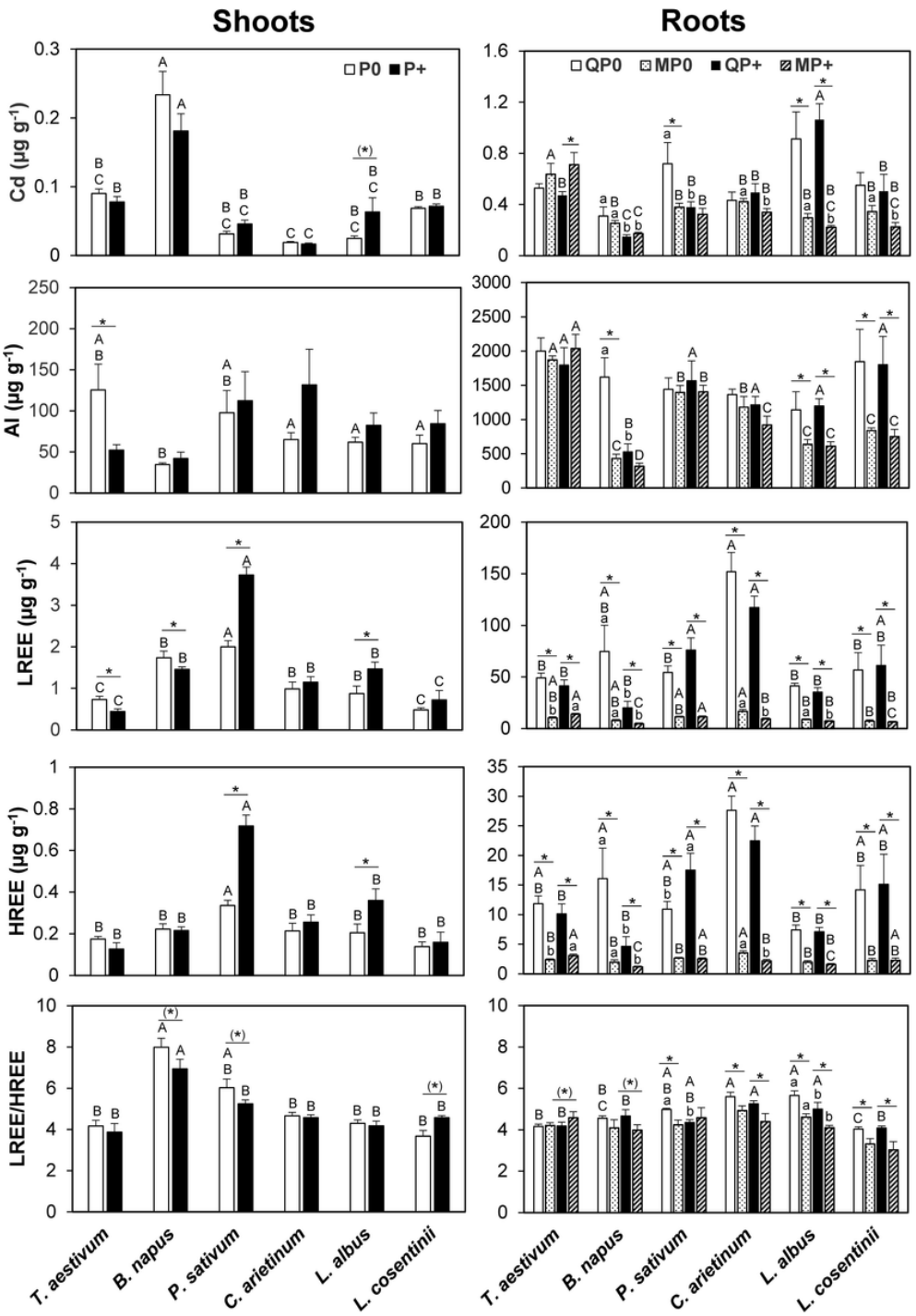

Figure 3

Concentrations of trace elements in shoots (left) and roots (right) of split-root plants treated without phosphors (low P) or with 100 µM 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 letters denote differences between P-treatments within a species and the root side. Additionally, in roots, asterisks indicate significant differences between root sides within a specific P-treatment at $p < 0.05$

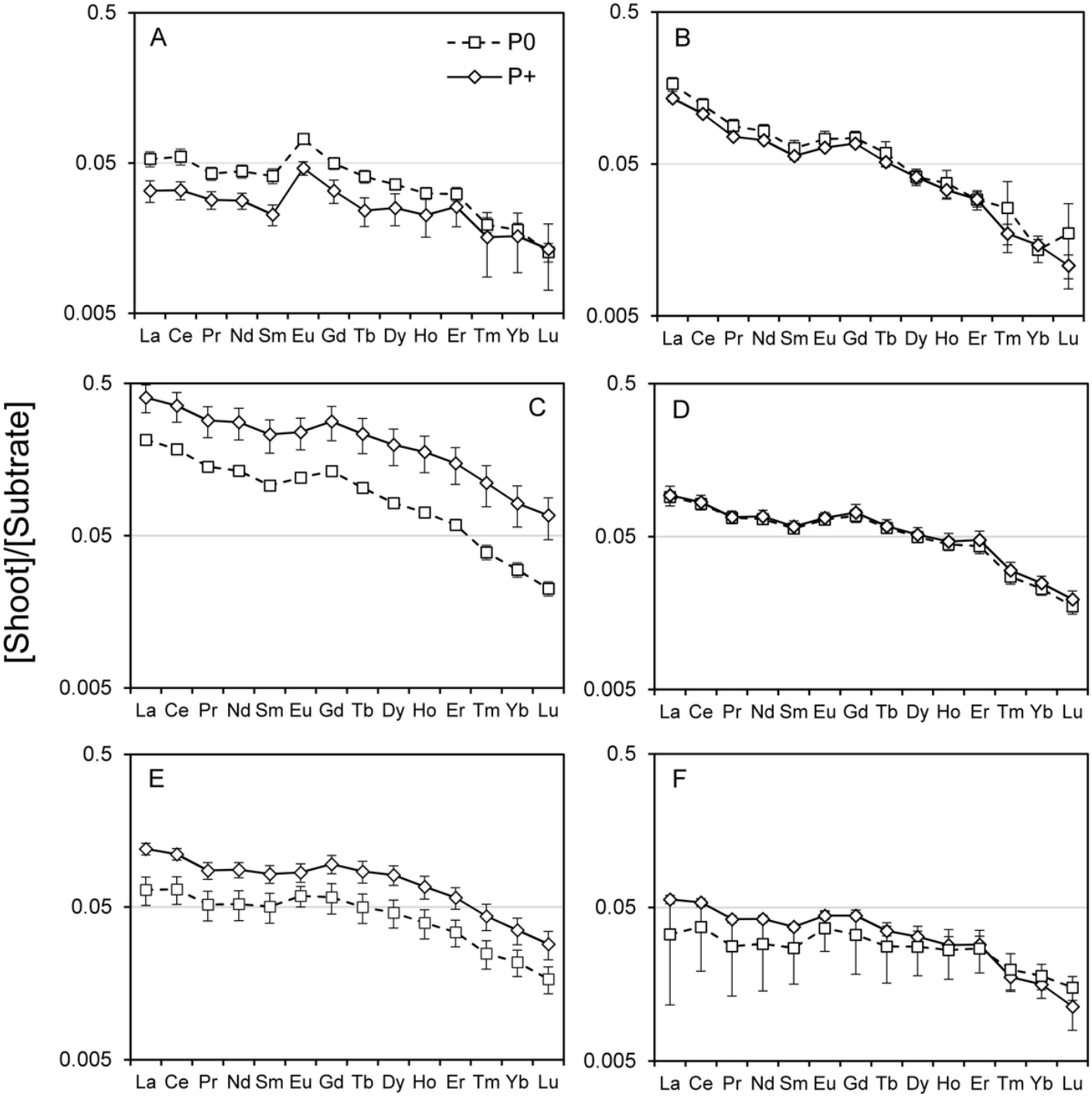

Figure 4

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) *Lupinus cosentinii* treated with 100 µM P (P+) or no P (0P)

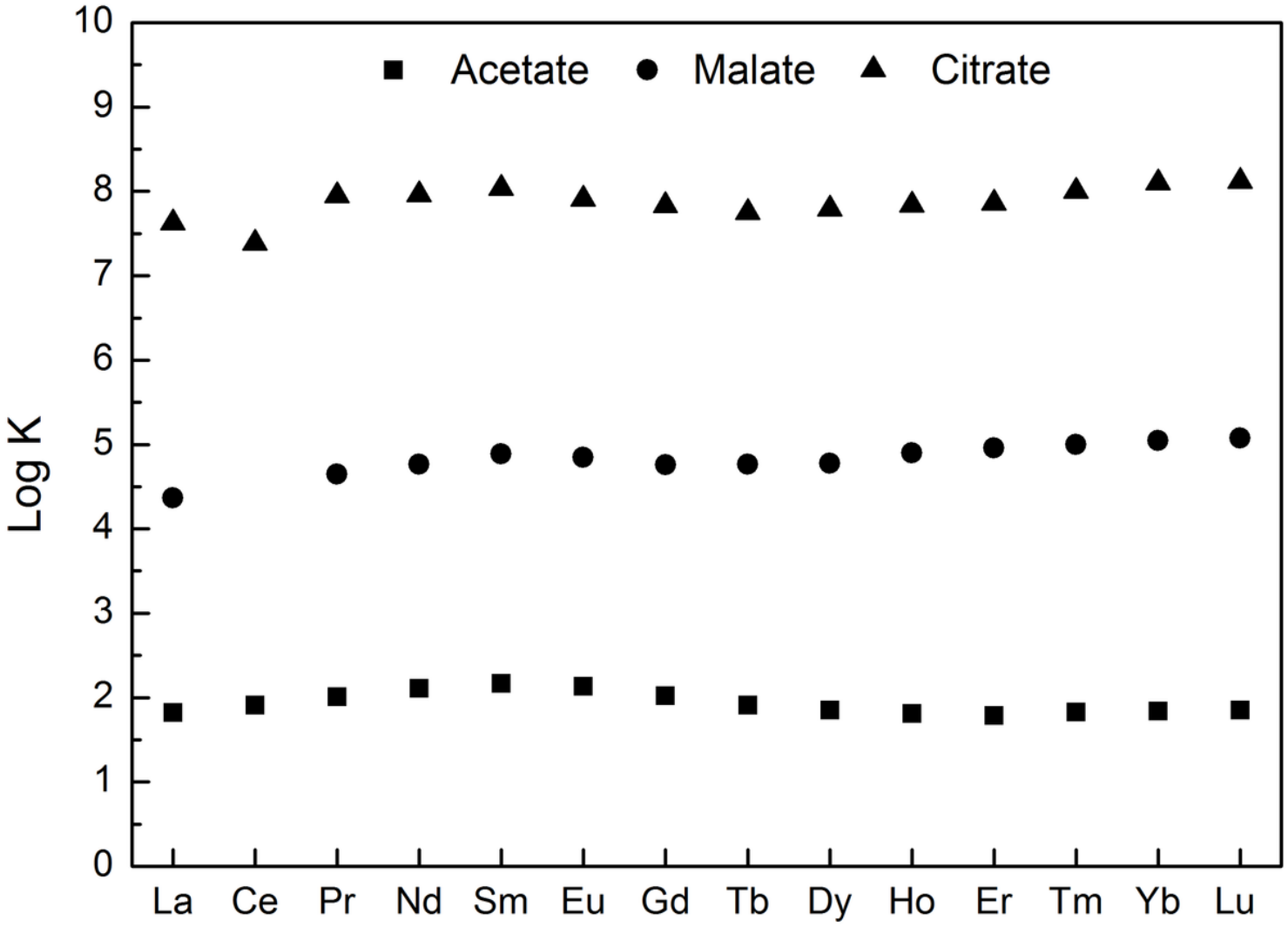

Figure 5

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