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REGULAR ARTICLE

Effects of pH and bicarbonate on the nutrient status and growth of three *Lupinus* species

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Abstract

Aims High pH, and high bicarbonate (HCO_3^-) and calcium (Ca) availability characterise calcareous soils. High [Ca] only partially explains why some *Lupinus* species are calcifuge, so we explored high [HCO_3^-] and high pH.

Methods We grew six *Lupinus* genotypes in hydroponics with pH 5, 6.5 and 8^a (adjusted by KOH), and 8^b (adjusted by $KHCO_3$). Leaf symptoms and areas, root appearance and biomass were recorded; whole leaf and root nutrient concentrations, and leaf cellular phosphorus (P), Ca and potassium (K) concentrations were determined using elemental X-ray microanalysis.

Results Chlorosis was observed in young leaves at high pH for *L. angustifolius* and *L. cosentinii*, and P deficiency at high pH for all genotypes. High pH decreased iron (Fe) and zinc (Zn) uptake in all genotypes. It also decreased lateral root growth, the uptake of P, K, Ca, and manganese (Mn) by all sensitive species; and translocation of P, Fe, Zn, Mn, and Ca to leaves in most sensitive species. However, leaf [Ca], leaf [K], [K] within each measured cell type, and translocation of K and Ca to leaves of *L. pilosus* and *L. cosentinii* at pH 8 were greater than at pH 5 and 6.5. Compared with $pH\ 8^a$, all *L. angustifolius* genotypes translocated more P, Fe, Zn, Mn and K from roots to leaves at $pH\ 8^b$. High pH did not affect the leaf cell types that accumulated P and Ca, but decreased the leaf cellular [P].

Conclusions *Lupinus angustifolius* and *L. cosentinii* were sensitive to high [HCO_3^-] and/or high pH; *L. pilosus* was relatively tolerant. High pH decreased lateral root growth and nutrient uptake, inhibiting growth of sensitive species. High [HCO_3^-] diminished the negative effect of pH 8 on nutrient translocation to leaves in most *L. angustifolius* genotypes. This knowledge provides critical insights into the habits of *Lupinus* species to guide breeding of calcicole plants.

Keywords High pH · High bicarbonate · Lateral root growth · Leaf chlorosis · Phosphorus deficiency · X-ray microanalysis

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Introduction

The calcifuge vs calcicole syndrome has been known for many decades (Tansley 1917) and explained in term of pH or pH-buffering capacity. Some plant species avoid calcareous soils (calcifuges) as opposed to preferring such soils (calcicoles), whereas some are soil-indifferent (Bugbee and Salisbury 1985; De Silva 1934; Zohlen and Tyler 2000). Many *Lupinus* species prefer acid soils rather than alkaline, calcareous soils (White 1990). This is remarkable, since they have the capacity to release carboxylic acids and strongly acidify their rhizosphere (Lambers et al. 2013; Neumann et al. 2000). High pH, and high bicarbonate (HCO_3^-) and calcium (Ca) availability are characteristics of calcareous soils, and are presumably the main factors explaining why some *Lupinus* species grow poorly on calcareous soils (Jessop et al. 1990; Tang and Robson 1995; Tang et al. 1995; Tang and Thomson 1996; White 1990). Some calcifuge *Lupinus* species show signs of Ca toxicity when grown at a high Ca supply (De Silva et al. 1994; Ding et al. 2018b; Jessop et al. 1990). *Banksia* and *Hakea* species (Proteaceae) are particularly sensitive to high Ca supply, when combined with a high phosphorus (P) supply (Nichols and Beardsell 1981). This is associated with the cellular allocation of P and Ca in their leaves. However, in our previous study, we found that a high Ca supply only partly explains the calcifuge habit of some *Lupinus* species. Therefore, other factors, such as high pH and high [HCO_3^-], need to be explored (Ding et al. 2018b).

Soil solution pH influences nutrient availability for root uptake (Neumann and Römheld 2012; White and Broadley 2009). At high pH (>7), the availability of P, iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and boron (B) is very low. As the pH decreases, the availability of Fe, Mn, Zn, B and aluminium (Al) increases due to increased solubility, desorption, or reduction (George et al. 2012; Parker et al. 1999). However, if it is too acidic, P can be sorbed onto soil particles, and mobile cations, such as potassium (K), Ca, and magnesium (Mg) can be leached as well (Lambers et al. 2008). A low nutrient availability related with a high pH has been the classic explanation why soil pH is a limiting factor for calcifuge species, explaining why they are sensitive to calcareous soils (Tyler and Ström 1995).

High [HCO_3^-], which ranges from 0.4 to 3 mM in calcareous soil solutions depending on soil water content (Mengel et al. 1984), can raise the soil pH. It also acts as a strong pH-buffer, and causes leaf chlorosis, both in calcareous soils (Coulombe et al. 1984b; Mengel et al. 1984) and in nutrient solution (Coulombe et al. 1984a; Romera et al. 1992; Tang and Thomson 1996; Waters and Troupe 2012). Bicarbonate can either stimulate or decrease root ferric chelate reductase (FCR) activity, depending on Fe supply (Hsieh and Waters 2016). It also inhibits Fe absorption and translocation to leaves (Bertoni et al. 1992; Fleming et al. 1984; Karimi and Tari 2017; Lucena et al. 2007; Romera et al. 1991; Rutland and Bukovac 1971) and causes Fe immobilisation in leaves (Kosegarten et al. 1999; Mengel et al. 1994). Similarly, high [HCO_3^-] can affect the availability and uptake of Mn, Cu and Zn (Coulombe et al. 1984a; Dogar and Van Hai 1980; Yue Ao et al. 1987).

High [HCO_3^-] not only negatively affects nutrient status, but also inhibits root growth of calcifuge species (Lee and Woolhouse 1969; Peiter et al. 2001; Tang et al. 1993b). In a hydroponic experiment, Tang and Thomson (1996) found that high pH and [HCO_3^-] decreases root elongation of a calcifuge *Lupinus* species, *L. angustifolius*, and this response was even observed after exposing it to nutrient solution pH (≥ 6) for one hour (Tang et al. 1992). However, the most pH-tolerant *Lupinus* species, *L. pilosus*, produces more cluster roots at high [HCO_3^-] (Tang et al. 1996) which release carboxylates and allow *L. pilosus* to access P and other nutrients that are otherwise poorly available at high pH (Dinkelaker et al. 1989; Lambers et al. 2013). It is obvious that there is a relationship between root morphology and nutrient status in plants when they grow in calcareous soils or nutrient solution high in [HCO_3^-].

There have been a number of studies investigating the effect of high [HCO_3^-] and/or high pH on the growth of *Lupinus* species (Brand et al. 2000; Kerley and Huyghe 2002; Tang and Robson 1993; White and Robson 1989a). However, it is difficult to separate the effects of high [HCO_3^-] from those of high pH, and then distinguish the effect of high [HCO_3^-] itself from that of high pH. This is because high pH will inevitably increase [HCO_3^-]. The best way to distinguish the difference of high [HCO_3^-] itself and that of high pH is by comparing differences between high [HCO_3^-] together with high pH and high pH alone. Those studies investigating the effects of high [HCO_3^-] and/or high pH alone focused on root growth or leaf Fe deficiency, and the causal relationship of root growth and nutrient deficiency under high pH is not clear. More importantly, other

nutrients in *Lupinus* species were not assessed. Our early study showed that Ca supply has no effect on the leaf cell types where P and Ca are allocated (Ding et al. 2018b), but we do not know if high [HCO_3^-] and/or high pH can change these allocation patterns. Therefore, the aims of this study were to examine the effects of high [HCO_3^-] and/or high pH on root growth, nutrient availability, P- and Ca-allocation patterns, and their relationship in *Lupinus* species. We hypothesised that nutrient uptake of calcicole *L. pilosus* and calcifuge *L. angustifolius* and *L. cosentinii* will be altered differently by pH increase, giving useful insights on chlorosis tolerance mechanism. We also hypothesised that the pH increase resulting from high [HCO_3^-], rather than HCO_3^- itself, is the main limiting factor regarding the calcifuge habitat of some *Lupinus* species. In addition, we hypothesised that any nutrient deficiencies in plants grown at high pH will be as a result of impaired nutrient uptake, rather than the effects of high pH on nutrient availability.

Materials and methods

Plant growth

Three *Lupinus* species (six genotypes, *L. angustifolius* cv L. Mandelup, *L. angustifolius* L. P25741, *L. angustifolius* L. P26723, *L. angustifolius* L. P22721, *L. pilosus* Murr. P27440 and *L. cosentinii* Guss. P27225) (Table 1) were chosen. All seeds were obtained from the Australian Lupin Collection (Department of Primary Industries and Regional Development, Western Australia). The seeds of *L. pilosus* and *L. cosentinii* were scarified and then all seeds were sterilised in 5% (*v*/v) sodium hypochlorite for 20 mins and rinsed with deionised (DI) water at least three times. All seeds were then soaked in deionised (DI) water overnight and sown in pots filled with sterilised river sand in a glasshouse (20 °C/15 °C day/night) to germinate. Ten days later, seedlings of similar size were chosen and washed free of sand. Each seedling was fixed in the centre of a foam lid and two plants were immersed in a 4 L black plastic pot with continuously aerated nutrient solution of the following composition: 200 μM KNO_3, 10 μM KH_2PO_4, 100 μM $CaCl_2$, 54 μM $MgSO_4$, 0.24 μM $MnSO_4$, 0.1 μM $ZnSO_4$, 2.4 μM H_3BO_3, 0.03 μM Na_2MoO_4, 0.018 μM $CuSO_4$ and 10 μM Fe-Na. EDTA (Ding et al. 2018a, b). After two weeks of acclimation in the glasshouse, all plants were placed under treatment. The four treatments were, pH 5, 6.5, 8^a (high pH, low [HCO_3^-], around 0.52 mM, as calculated by Geochem-EZ (Shaff et al. 2010)), and 8^b (high pH, high [HCO_3^-], around 1.4 mM, as calculated by Geochem-EZ). Treatments pH 5, 6.5 and 8^a were adjusted by KOH and buffered with MES and TES (0.5 mM each). Treatment pH 8^b was adjusted by $KHCO_3$ with no MES or TES added, and the resulting [HCO_3^-] in the nutrient solution was 1.5 mM. The ion free activities of different treatments predicted by GeoChem-EZ are shown in Table S1. The average daily pH of the nutrient solution in which the six *Lupinus* genotypes were grown including manual pH adjustments were recorded (Fig. S1). In total, there were 6 genotypes (3 species) × 3 replicates (3 pots) × 2 plants in each pot × 4 pH treatment =144 plants. The pH adjusted by KOH (5, 6.5 and 8^a) was adjusted three times daily, early morning, noon and night. When the nutrient solutions were changed, [K^+] in all the treatments were balanced to be 1.71 mM. Between each nutrient solution change, [K^+] was not balanced, as the amount of KOH added to adjust the pH was hard to calculate. The pH of treatment pH 8^b was stable during

Table 1 *Lupinus* species and genotypes used in this study with collection site soil pH and origin, their calcicole and calcifuge habits are based on their natural occurrence and other studies (Brand et al. 1999; Gladstones 1974; Kerley 2000; White 1990)

Species	Genotype	Breeding status	Collection site soil pH	Country of origin	Calcicole/ Calcifuge
L. angustifolius	Mandelup	cultivar	unknown	Australia	calcifuge
L. angustifolius	P25741	naturalised	5	Spain	calcifuge
L. angustifolius	P26723	naturalised	6.5	Spain	calcifuge
L. angustifolius	P22721	naturalised	7.5	Spain	calcifuge
L. pilosus	P27440	naturalised	9.0	Syria	calcicole
L. cosentinii	P27225	naturalised	9.0	Morocco	calcicole

the experiment, so there was no need to adjust it. The nutrient solution was changed three times per week to minimise depletion of nutrients and bicarbonate accumulation. Pots were placed in a temperature-controlled, root-cooling tank maintained at 18 °C.

Gas exchange and chlorophyll fluorescence measurements

Net photosynthesis rate (A_{max}) and stomatal conductance (g_s) were measured on the youngest fully-expanded leaves using a LI-6400 portable gas exchange system (Li-Cor, Lincoln, NE, USA) at 1500 μmol quanta m^{-2} s^{-1}. Stomatal limitation (L_s) was calculated according to: $L_s=1-\frac{C_i}{C_a}$ (Jones 1985). The same leaf used to measure photosynthesis was dark-adjusted in a leaf clip for 30 min, and then the maximum fluorescence yield (F_m) and the minimum fluorescence yield (F_o) were measured by a Hansatech Plant Efficiency Analyser (PEA, King's Lynn, Norfolk, UK). The maximum photochemical quantum yield of PSII (F_v/F_m) was calculated according to $F_v/F_m = (F_m - F_o)/F_m$ (Maxwell and Johnson 2000).

Net P- and Ca-uptake rate

Four individual plants from all genotypes under each treatment were placed in a basal nutrient solution with no P or Ca added for >16 h (overnight) prior to measurement. The next day, the root surfaces of each plant were carefully blotted with paper towels and placed in a separate fresh 1 L nutrient solution with either 10 μM P or 0.1 mM Ca; the pH of the nutrient solution was kept the same in each treatment. A sample of solution (1 mL for P and 5 mL for Ca) was withdrawn every 30 mins for P, and every hour for Ca to determine the uptake rate, over a four-hour period. The solution was continuously aerated throughout the whole experiment. Phosphorus concentration in the solution was measured by the malachite green method (Motomizu et al. 1983), and [Ca] was measured by inductively coupled plasma optical emission spectrometry (ICP-OES; School of Agriculture and Environment, University of Western Australia, Perth, Australia). The [P] and [Ca] were then used to calculate the P- and Ca-depletion rates and thus the net P- and Ca-uptake rates per unit root dry weight for each plant.

Plant harvest

After five weeks of treatment, plants were carefully and thoroughly washed with deionised (DI) water. Plants were then separated into mature leaves (totally expanded leaves), immature leaves (partly expanded leaves), stems (including petioles) roots (including non-cluster and cluster roots). Cluster roots, also called proteoid roots, comprise many lateral rootlets (0.5–35 mm long) along a root axis of 0.5–4 cm (Dinkelaker et al. 1995; Shane and Lambers 2005). Leaf area was measured by a Li-Cor Ll- 3000 leaf area meter with a LI-3050A transparent belt conveyor (Li-Cor, Lincoln, NE, USA). Dry weights of all the plant parts were recorded after drying them in an oven at 70 °C for one week. Leaf symptoms and root visual appearance were recorded by taking photos prior to harvest.

Whole leaf nutrient concentrations, leaf + root nutrient content and root-to-leaf nutrient translocation ratio

Dry leaves and roots were ground into a fine powder using plastic vials and ceramic beads in a GenoGrinder vertical ball mill (SPEX SamplePrep LLC, Metuchen, New Jersey, USA). A subsample of 100 mg dried ground material was then digested with concentrated HNO_3 and $HClO_4$ (3:1) and the concentration of Ca, Cu, Fe, Mg, Mn, P, S, and Zn determined using inductively coupled plasma optical emission spectrometry (ICP-OES). Leaf and root nutrient content was calculated as leaf and root nutrient concentration × leaf and root dry biomass. Leaf + root nutrient content was calculated as leaf nutrient content + root nutrient content; the root-to-leaf ratio was expressed as the ratio (%) of leaf nutrient content to leaf + root nutrient content (Impa et al. 2013).

Distribution of P, Ca and K within leaf cells

Prior to harvest, a small section (~ 3 × 3 mm) was cut from the youngest fully-expanded leaves of each treatment to measure leaf cellular P, Ca and K concentrations, which are average P, Ca and K concentrations (based on fresh weight) in each leaf cell (including all the organelles and cytosol). Samples were then carefully stored and prepared to be measured with X-ray microanalysis; the details of sample preparation and analytical system can be found in Ding et al. (2018b). This method has been shown to be highly suitable for cellular element

analyses of biological samples (Guilherme Pereira et al. 2018; Hayes et al. 2018; Huang et al. 1994; Marshall 2017; McCully et al. 2010).

For cellular analyses, oblique cells and airspaces were avoided and only cells that were clearly identifiable and had a flat surface were chosen. Different *Lupinus* species possess similar anatomy and cell types as noted before (Ding et al. 2018b), including upper epidermal cells (UE), palisade mesophyll cells (PM), spongy mesophyll cells (SM), lower epidermal cells (LE), bundle sheath cells, and veins (Fig. S2). The concentrations of P, Ca, and K in UE, PM, SM, and LE were thus obtained by selecting these cells in the elemental maps and quantifying the resulting summed spectra from each region of interest.

Statistics

Data were analysed with the R software platform (R Core Team 2017). General linear mixed-effect models were used to test the differences in nutrient-uptake rate, nutrient concentrations, leaf area, and biomass among species, pH and the interaction between them. We tested the differences of gas exchange parameters and chlorophyll fluorescence with general linear mixed-effect models among species, pH and the interaction between these two factors. Differences in leaf cell [nutrient] were tested using general linear mixed-effect models among different pH, cell types and the interactions of treatments and cell types, with individual plants as the random effect. Based on Akaike Information Criterion (AIC), models with different variance structures were compared to find the one with the residuals most closely meeting homoscedasticity (Burnham and Anderson 2003; Zuur et al. 2009). The mean values and 95% confidence intervals (Cl) were determined by the effects package and plotted (Fox 2003). The significant differences were defined based on Tukey's post-hoc analysis ($P < 0.05$). Model results ($P < 0.05$) are shown in Table S2-S6.

Results

Leaf symptoms

Leaf chlorosis was observed in the youngest leaves at pH 8 (both 8.0^{a}, i.e. adjusted by KOH, and 8.0^{b}, i.e. adjusted by $KHCO_3$) for all *L. angustifolius* genotypes and *L. cosentinii*, but it was less severe for *L. cosentinii*. Interestingly, leaf chlorosis was much worse for all *L. angustifolius* genotypes at pH 8.0^{a} than at 8.0^{b} (Fig. 1). We observed P-deficiency symptoms at pH 8 for all genotypes, with older leaves of *L. angustifolius* wild genotypes exhibiting a red colour, and older leaves of *L. angustifolius* cv, *L. cosentinii* and *L. pilosus* exhibiting a yellow colour first, and then a dried appearance (Figs. 1 and S3).

Root appearance

The lateral root growth of all genotypes was inhibited at higher pH, but the least for *L. pilosus*. In addition, root disintegration was observed in all of the *L. angustifolius* genotypes at pH 8 (Fig. 2).

Net P- and Ca-uptake rates

The net P-uptake rates of all *L. angustifolius* genotypes and *L. cosentinii* were significantly and negatively affected at higher pH, while pH had no effect on the P-uptake rate of *L. pilosus*. The net Ca-uptake rate of calcifuge *L. angustifolius* cv Mandelup was significantly faster at pH 8 than that at pH 5 and 6.5, whereas pH only had a minor effect on the net Ca-uptake rate of calcicole *L. pilosus* (Fig. 3).

Whole leaf nutrient concentrations

Compared with those at pH 5, leaf [P], [Fe] and [Zn] at pH 8 were significantly lower for all genotypes; leaf [P] of all genotypes at pH 8 was below the concentration considered deficient for whole shoots for *L. albus* (1.3 mg g^{-1}) (Loneragan and Snowball 1969); leaf [P] of all *L. angustifolius* genotypes and leaf [Zn] of all genotypes at pH 6.5 were also significantly lower (Fig. 4a, b and c). Compared with that at pH 5, leaf [Mn] of all *L. angustifolius* wild genotypes was significantly lower at pH 8.0^{b}, whereas there was no significant difference for *L. angustifolius* cv Mandelup, *L. pilosus* and *L. cosentinii*; however, leaf [Mn] of all genotypes was significantly lower at pH 8.0^{a} (Fig. 4d). Only some leaf [Mn] of *L. angustifolius* genotypes at pH 5 and 6.5 were around the critical shoot [Mn] (277 mg g^{-1}); in other words, meeting the requirement for near-maximum growth (Snowball and Robson 1986). Compared with that at pH 5 and 6.5, leaf [K] of *L. angustifolius* cv Mandelup and *L. angustifolius*

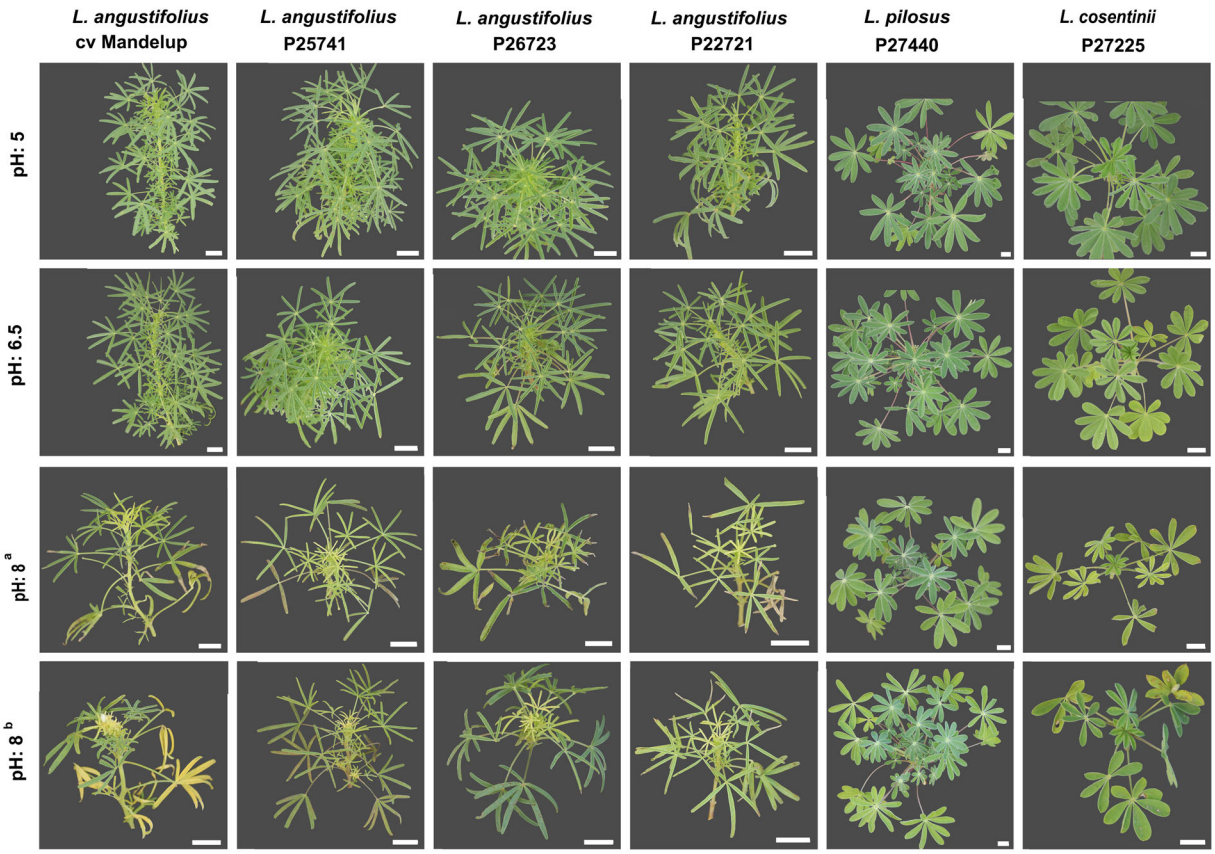


Fig. 1 Leaf symptoms of six *Lupinus* genotypes when grown at different pH. The pH 5, 6.5 and 8[a] were adjusted by KOH and buffered with MES and TES (0.5 mM each), whereas 8[b] was adjusted by $KHCO_3$ without MES or TES added. Scale bars are 2 cm

P22721 were significantly lower at pH 8, while those of *L. pilosus* and *L. cosentinii* were significantly higher; pH had no effect on the leaf [K] of *L. angustifolius* P25741 and P26723 (Fig. 4e). Among all pH treatments, leaf [Ca] of all *L. angustifolius* genotypes at pH 6.5 were the highest, while those of *L. pilosus* and *L. cosentinii* were greater at higher pH (Fig. 4f). Leaf Mg, Cu and sulfur (S) concentrations are shown in Fig. S4, and root nutrient concentrations in Fig. S5.

Leaf + root nutrient content

Compared with those at pH 5 and 6.5, total P content of all *L. angustifolius* genotypes and *L. cosentinii* were significantly lower at pH 8, while total P content of *L. pilosus* at pH 8 showed no significant difference with that at pH 6.5 (Fig. 5a). Total Fe and Zn content of all the genotypes showed a similar trend with total P content (Fig. 5b and c). Compared with those at pH 5 and 6.5, total Mn content of all the *L. angustifolius* genotypes was significantly lower at pH 8, while total Mn content of *L. cosentinii* at pH 8.0[b] showed no significant difference with those at pH 5 and 6.5. The total Mn content of *L. pilosus* showed no significant differences between different treatments (Fig. 5d). Total K and total Ca content of all the genotypes showed a similar trend with total Mn content (Fig. 5e and f). Total Mg, Cu and S contents are shown in Fig. S6. Whole leaf and root nutrient contents are shown in Figs. S7 and S8.

Root-to-leaf nutrition translocation ratio

For all the *L. angustifolius* genotypes and *L. cosentinii*, when the pH was higher, the P translocation index tended to be lower, while there was no significant difference for *L. pilosus* among different treatments. The Mn translocation ratios of all the species also tended to be lower at pH 8 than those at pH 5 and 6.5. The Fe translocation ratios of *L. angustifolius* genotypes (except *L. angustifolius* P22721) and *L. cosentinii* at pH 6.5

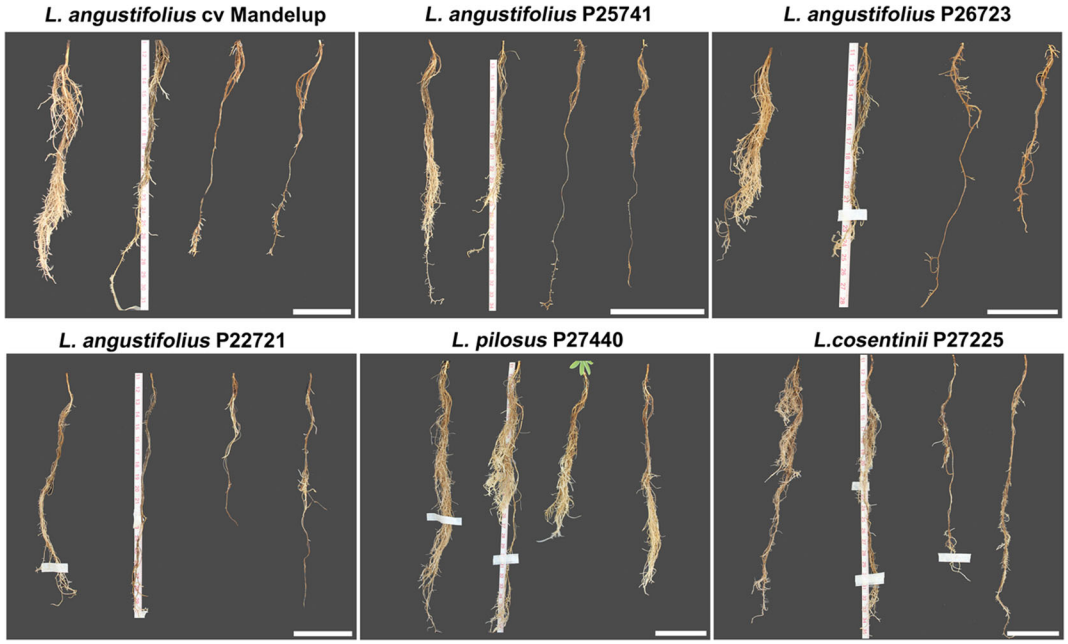


Fig. 2 Root morphology of six *Lupinus* genotypes when grown with different pH. From left to right in each photo, the pH is 5, 6.5, 8^a and 8^b individually. The pH 5, 6.5 and 8^a were adjusted by KOH and buffered with MES and TES (0.5 mM each), whereas 8^b was adjusted by $KHCO_3$ without MES or TES added. Scale bars are 5 cm

were significantly lower than those at pH 5; and the trend for the Zn translocation indices were very similar to those of Fe translocation ratios. The trend of K and Ca translocation ratios for all the *L. angustifolius* genotypes were not very clear, but for *L. cosentinii* and *L. pilosus*, the K and Ca translocation ratios at pH 8 were significantly higher than those at pH 5 and 6.5 (Table 2).

For all the *L. angustifolius* genotypes, the P, Fe, Zn, Mn and K translocation ratios at pH 8.0^b were generally higher than those at pH 8^a (Table 2). The other nutrient (Mg, Cu and S) root-to-leaf translocation indices are shown in Table S7.

Leaf cellular nutrient concentration in different cell types

In all genotypes, most P was located in epidermal cells (both UE and LE) at pH 5 and 6.5, while [P] in all cell types were very low and largely consistent across all cell types at pH 8. The [P] in the epidermal cells (both UE and LE) were significantly lower at pH 8 than at pH 5 and 6.5 (Fig. 6a).

For all genotypes, most Ca accumulated in the mesophyll cells and tended to accumulate more in PM than SM. The [Ca] in the mesophyll cells of *L. angustifolius* cv Mandelup were significantly higher at pH 8 than at pH 5 and 6.5. The [Ca] in the PM of *L. angustifolius* P26723 were higher at pH 6.5 and pH 8.0^a than at pH 5, while they did not differ for SM among these pH treatments; however, it was the lowest for both PM and SM at pH 8.0^b among all pH treatments. The [Ca] in PM of *L. pilosus* at pH 6.5 and 8.0^a were significantly higher than those at pH 5 and 8.0^b, while the [Ca] in SM at pH 8.0^a were the highest among all pH treatments. The [Ca] in PM of *L. cosentinii* at pH 8.0^a were the lowest among all pH treatments, while they were the highest at pH 8.0^b. The [Ca] in the SM of *L. cosentinii* were higher at higher pH (Fig. 6b).

At pH 5 and 6.5, *L. angustifolius* cv Mandelup tended to accumulate more K in epidermal cells; at pH 8, more K was allocated to LE and SM. In *L. angustifolius* P26723, at pH 5, 6.5 and 8.0^b, mesophyll cells tended to accumulate more K than epidermal cells. However, at pH 8.0^a, LE and SM of *L. angustifolius* P26723 were the cell types that exhibited the largest [K], followed by PM, then UE. *Lupinus pilosus* and *L. cosentinii* accumulated more K in mesophyll cells at all pH treatments. Cellular [K] of *L. pilosus* and *L. cosentinii* at pH 8 were much higher than at pH 5 and 6.5 for all cell types (Fig. 6c). Cellular Mg, S and chlorine (Cl) concentrations are shown in Fig. S9.

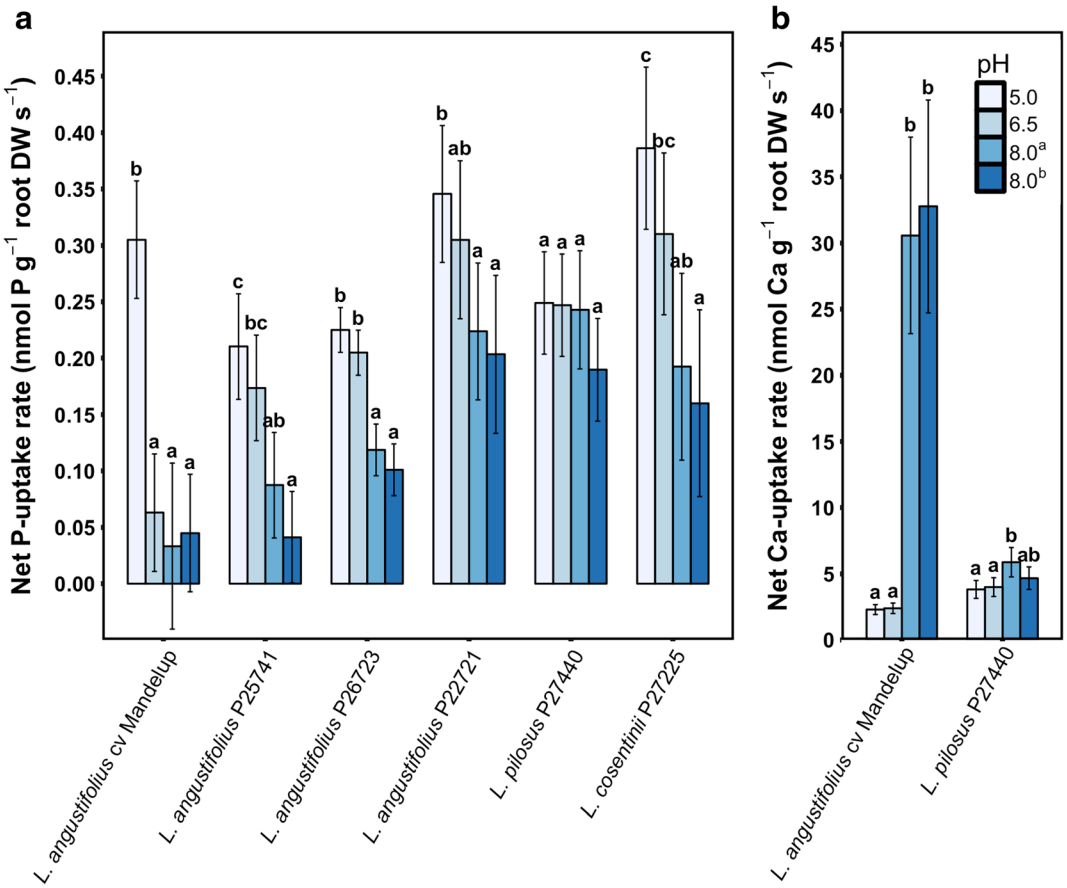
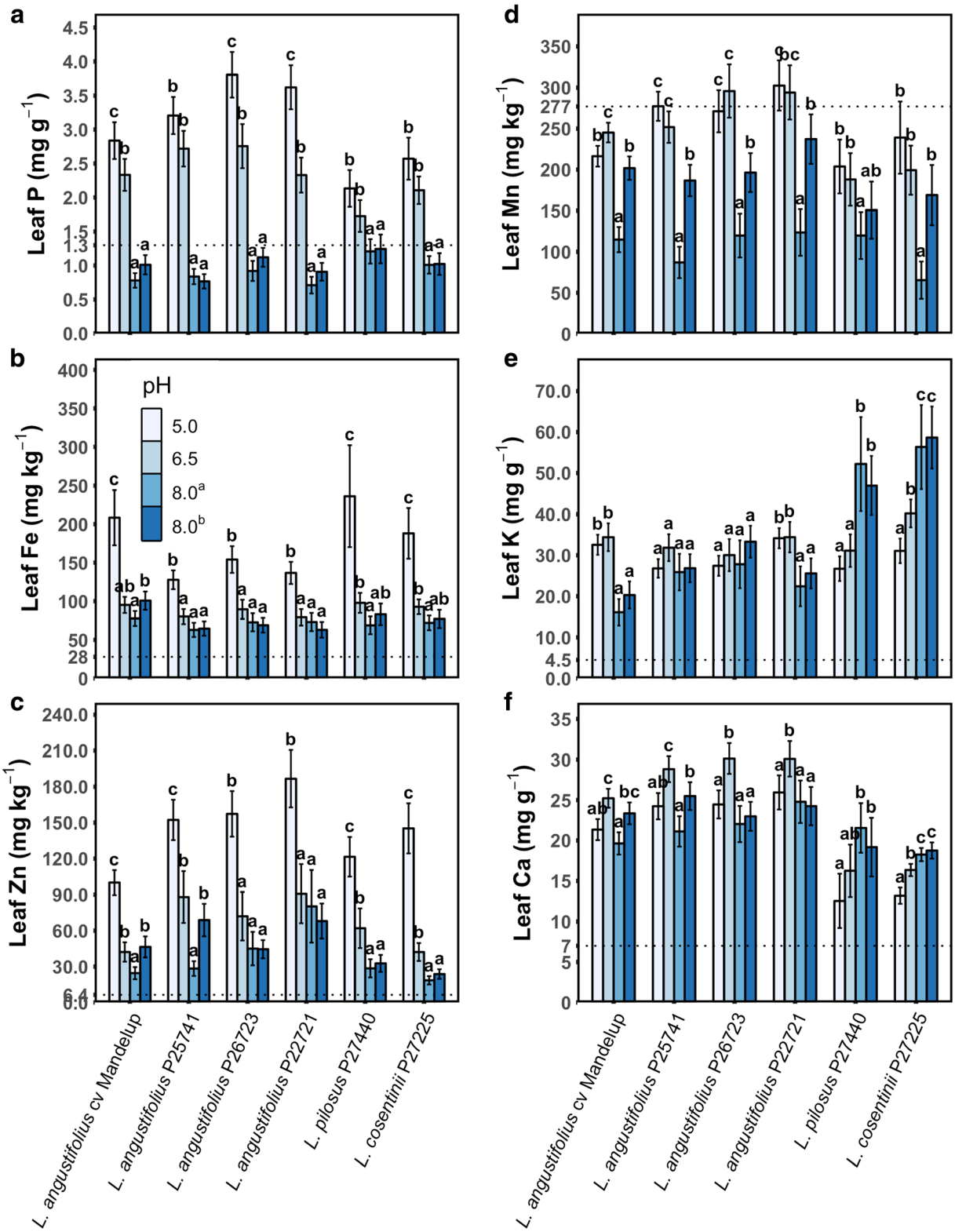


Fig. 3 Net phosphorus (P)-uptake rate of six *Lupinus* genotypes (**a**) and calcium (Ca)-uptake rate of two *Lupinus* genotypes, one calcifuge and one calcicole genotype (**b**) when grown at different pH. The pH 5, 6.5 and 8^{a} were adjusted by KOH and buffered with MES and TES (0.5 mM each), whereas 8^{b} was adjusted by $KHCO_3$ without MES or TES added. The rates shown were calculated from P- and Ca-depletion in the external solution, which was the same for all plants, irrespective of prior growing conditions; for details, refer to Materials and Methods. Error bars represent 95% confidence intervals (Cl). Letters show significant differences of different pH within each genotype (based on Tukey's post-hoc analysis, $P < 0.05$)

Gas exchange and chlorophyll fluorescence

Net photosynthetic rates of all genotypes at pH 8.0^{a} were significantly slower than those at pH 5 and 6.5, and were even slower than those at pH 8.0^{b} for *L. angustifolius* P25741 and P22721, *L. pilosus* and *L. cosentinii*. Rates of all *L. angustifolius* genotypes and *L. cosentinii* at pH 8.0^{b} were significantly lower than those at pH 5 and 6.5, with no significant difference for *L. pilosus* (Fig. 7a). Stomatal conductance (g_s) of all genotypes at pH 8.0^{a} was the lowest, except for *L. angustifolius* cv Mandelup, showing the smallest g_s at pH 8.0^{b}; there was a large difference in g_s for all genotypes between pH 8.0^{a} and 8.0^{b} (Fig. 7b). Stomatal limitation (L_s) of all *L. angustifolius* genotypes at pH 8.0^{b} and L_s of all the *L. angustifolius* wild genotypes

Fig. 4 Concentrations of a range of nutrients in whole leaves of six *Lupinus* genotypes when grown at different pH. The pH 5, 6.5, and 8^{a} were adjusted by KOH and buffered with MES and TES (0.5 mM each), whereas 8^{b} was adjusted by $KHCO_3$ without MES or TES added. Error bars represent 95% confidence intervals (Cl). Letters show significant differences of different pH within each genotype (based on Tukey's post-hoc analysis, $P < 0.05$). The grey dashed line represents the deficient (P, Ca, K, Fe, Zn) or critical (Mn) nutrient concentration in dry matter of whole shoots or pairs of youngest open leaves (YOL) of *L. angustifolius* or *L. albus* harvested on different dates; critical concentration means that the concentration required for maximum growth (Snowball and Robson 1986)


a
Leaf P (mg g^{-1})
b
Leaf Fe (mg kg^{-1})
pH
5.0
6.5
8.0^{a}
8.0^{b}
c
Leaf Zn (mg kg^{-1})
d
Leaf Mn (mg kg^{-1})
e
Leaf K (mg g^{-1})
f
Leaf Ca (mg g^{-1})
L. angustifolius cv Mandelup
L. angustifolius P25741
L. angustifolius P26723
L. angustifolius P22721
L. pilosus P27440
L. cosentinii P27225

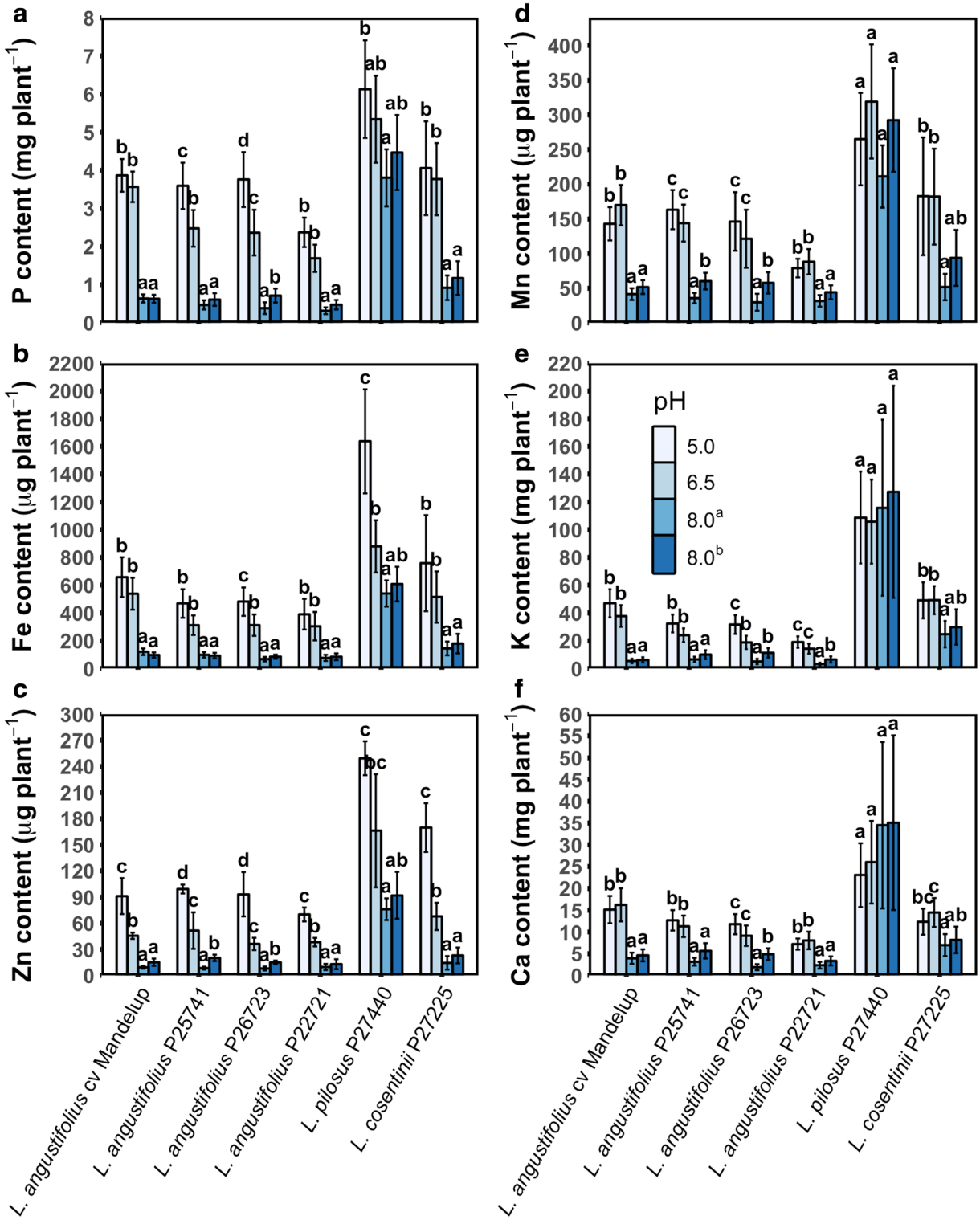


Fig. 5 Leaf + root nutrient content of six *Lupinus* genotypes when grown at different pH. The pH 5, 6.5 and 8^{a} were adjusted by KOH and buffered with MES and TES (0.5 mM each), whereas 8^{b} was adjusted by $KHCO_3$ without MES or TES added. Error bars represent 95% confidence intervals (Cl). Letters show significant differences of different pH within each genotype (based on Tukey's post-hoc analysis, $P < 0.05$)

Table 2 Root-to-leaf nutrient translocation ratio (mean ± 95% confidence intervals) of different *Lupinus* species when grown at different pH

Species	pH	P	Fe	Zn	Mn	K	Ca
L. angustifolius cv Mandelup	5	41 ± 1d	19 ± 1b	63 ± 2b	85 ± 2d	40 ± 2a	80 ± 1a
	7	34 ± 2c	9 ± 1a	46 ± 33a	74 ± 2c	48 ± 3ab	80 ± 1a
	8^a	16 ± 2a	9 ± 1a	35 ± 5a	39 ± 5a	40 ± 2a	72 ± 4a
	8^b	25 ± 2b	16 ± 1b	48 ± 3a	60 ± 3b	53 ± 4b	81 ± 2a
L. angustifolius P25741	5	39 ± 1b	13 ± 1b	65 ± 2b	74 ± 2c	36 ± 1a	83 ± 1b
	7	36 ± 2b	9 ± 1a	59 ± 3b	58 ± 3b	44 ± 2b	85 ± 1b
	8^a	19 ± 2a	7 ± 1a	37 ± 5a	27 ± 6a	44 ± 2b	74 ± 3a
	8^b	24 ± 2a	13 ± 1b	64 ± 3b	58 ± 4b	52 ± 3b	85 ± 1b
L. angustifolius P26723	5	39 ± 1c	13 ± 1b	65 ± 2b	73 ± 3c	34 ± 1b	82 ± 1a
	7	30 ± 2b	7 ± 1a	50 ± 3a	63 ± 4bc	41 ± 2c	84 ± 1a
	8^a	13 ± 2a	6 ± 2a	36 ± 7a	25 ± 9a	28 ± 1a	64 ± 14a
	8^b	27 ± 2b	14 ± 1b	50 ± 3a	60 ± 4b	53 ± 2d	82 ± 1a
L. angustifolius P22721	5	35 ± 1c	9 ± 1a	60 ± 2a	86 ± 1	41 ± 3a	82 ± 1a
	7	28 ± 2b	6 ± 1a	52 ± 3a	79 ± 2c	53 ± 6a	86 ± 1b
	8^a	15 ± 2a	7 ± 1a	57 ± 6a	26 ± 2a	49 ± 5a	73 ± 4a
	8^b	20 ± 2a	8 ± 1a	52 ± 3a	53 ± 3b	45 ± 4a	78 ± 2a
L. pilosus P27440	5	45 ± 2a	17 ± 2ab	63 ± 3a	94 ± 1b	35 ± 3a	74 ± 1a
	7	44 ± 2a	15 ± 1a	56 ± 4a	80 ± 1a	39 ± 3a	79 ± 1b
	8^a	44 ± 4a	18 ± 1ab	53 ± 6a	79 ± 1a	66 ± 5b	90 ± 1d
	8^b	42 ± 3a	21 ± 2b	53 ± 4a	78 ± 1a	56 ± 5b	85 ± 1c
L. cosentinii P27225	5	46 ± 1c	18 ± 1b	62 ± 2b	95 ± 2d	46 ± 2a	78 ± 1a
	7	39 ± 2b	13 ± 1a	42 ± 3a	76 ± 2c	59 ± 2b	80 ± 1ab
	8^a	35 ± 3ab	15 ± 1ab	40 ± 5a	37 ± 5a	74 ± 3c	84 ± 1c
	8^b	30 ± 2a	16 ± 1ab	35 ± 3a	62 ± 4b	70 ± 3c	83 ± 1bc

The pH 5, 6.5 and 8^a were adjusted by KOH and buffered with MES and TES (0.5 mM each), whereas 8^b was adjusted by $KHCO_3$ without MES or TES added. Letters show significant differences of different pH within each genotype (based on Tukey's post-hoc analysis, $P < 0.05$)

at pH 8.0^a were significantly higher than at pH 5 and 6.5, while L_s of *L. angustifolius* cv Mandelup did not differ between pH 5, 6.5 and 8.0^a. Stomatal limitation of *L. pilosus* at pH 8.0^a was the highest among all pH treatments, with no significant difference for *L. cosentinii* (Fig. 7c). *Fv/Fm* of all genotypes at pH 8 were smaller than those at pH 5 and 6.5 (Fig. 7d).

Biomass

Leaf area of all *L. angustifolius* genotypes and *L. cosentinii* were negatively affected by pH 8, and that of *L. angustifolius* P26723 was less at pH 6.5, with no effect for *L. pilosus* (Fig. 8a). A similar trend was found for shoot and total root biomass (Fig. 8b and c). The two cluster-root forming species, *L. pilosus* and *L. cosentinii*, showed no significant differences in cluster-root biomass among pH treatments (Fig. 8d).

Discussion

All *L. angustifolius* genotypes and *L. cosentinii* P27225 were sensitive to pH 8 (both 8.0^a, i.e. adjusted by KOH, and 8.0^b, i.e. adjusted by $KHCO_3$), while *L. pilosus* P27440 was relatively tolerant. The growth of all pH-sensitive genotypes was significantly less at pH 8 than that at pH 5 and 6.5, while the growth of the pH-tolerant genotype did not differ among pH treatments. All *L. angustifolius* genotypes and *L. cosentinii* exhibited P-deficiency and leaf chlorosis symptoms, while *L. pilosus* only exhibited P-deficiency symptoms at pH 8.

The effect of pH on root morphology

A high $[HCO_3^-]$ and/or high pH negatively affects the root growth of *L. angustifolius* and *L. albus* (Kerley and

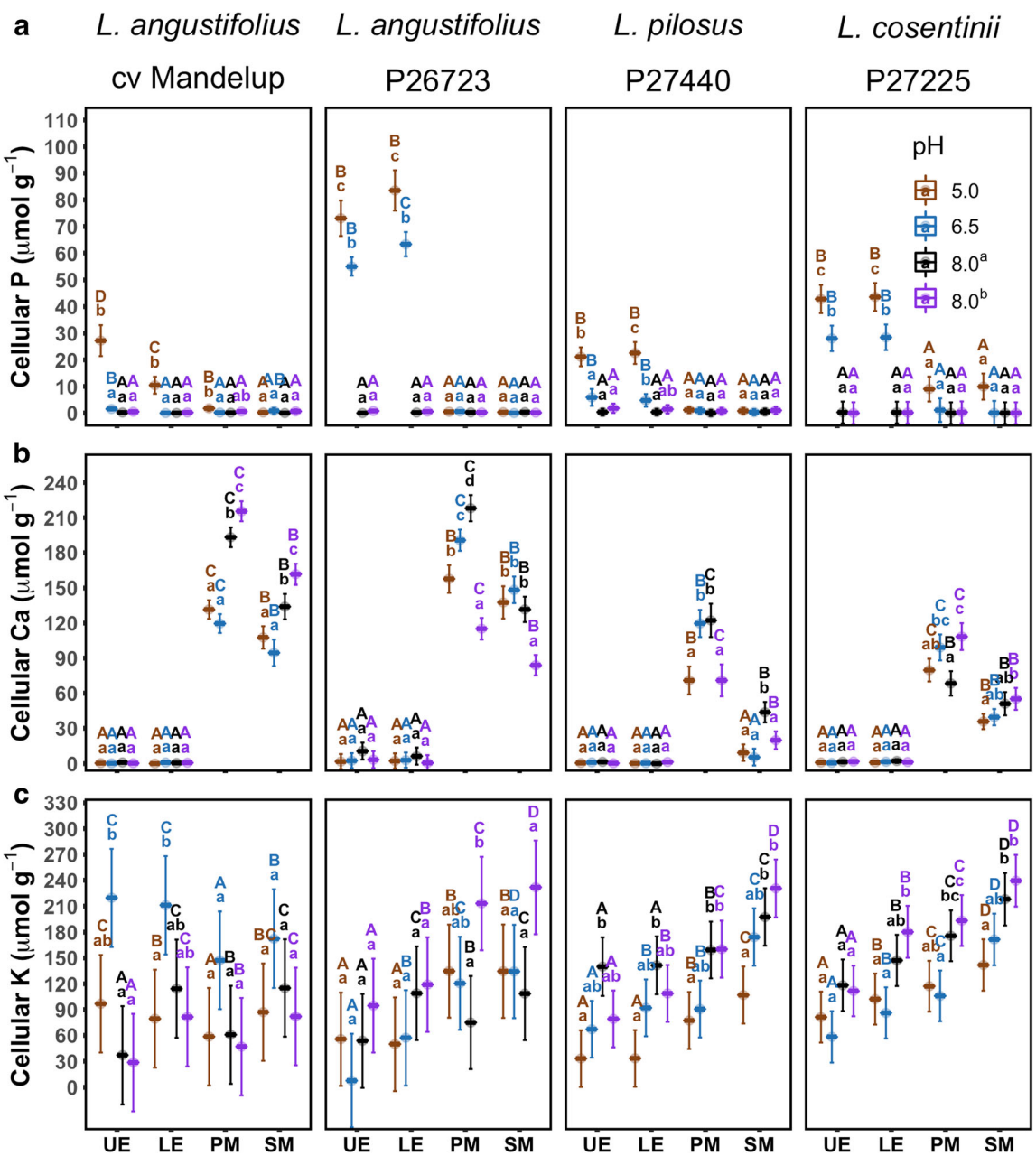


Fig. 6 Concentrations of phosphorus (P) (**a**) calcium (Ca) (**b**) and potassium (K) (**c**) in individual leaf cell types of four *Lupinus* genotypes when grown at different pH. The pH 5, 6.5 and 8^a were adjusted by KOH and buffered with MES and TES (0.5 mM each), whereas 8^b was adjusted by $KHCO_3$ without MES or TES added. Error bars represent 95% confidence intervals. UE, upper epidermal cells; LE, lower epidermal cells; PM, palisade mesophyll cells; SM, spongy mesophyll cells. Uppercase letters show significant differences among different cell types at the same pH, while lowercase letters show significant differences at different pH within each genotype (based on Tukey's post-hoc analysis, $P < 0.05$)

Huyghe 2002; Tang et al. 1996; Tang and Robson 1993; White and Robson 1990). In this study, the lateral root growth of all genotypes was inhibited at pH 8, but it was least severe for *L. pilosus*. In addition, root disintegration was observed in the *L. angustifolius* genotypes at pH 8, and this confirmed what Tang et al. (1993a) found. Decreased root elongation has also been observed in *L. angustifolius* in alkaline soils (Tang et al. 1992;

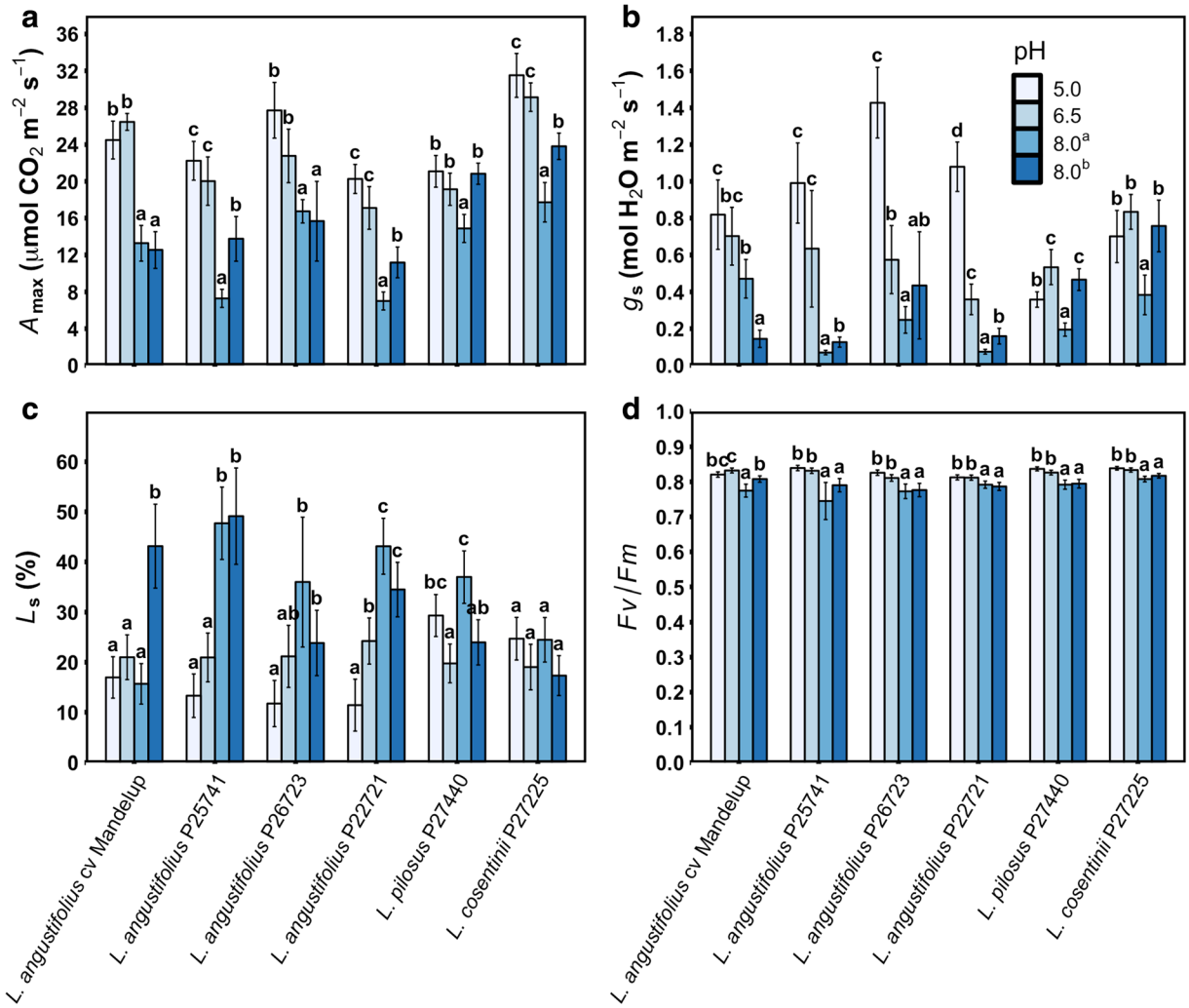


Fig. 7 Net photosynthetic rate (A_{max}) (**a**), stomatal conductance (g_s) (**b**), stomatal limitation (L_s) (**c**), and *Fv/Fm* (**d**) of six *Lupinus* genotypes when grown with different pH. The pH 5, 6.5 and 8^a were adjusted by KOH and buffered with MES and TES (0.5 mM each), whereas 8^b was adjusted by $KHCO_3$ without MES or TES added. Error bars represent 95% confidence intervals. Letters show significant differences between different pH within each genotype (based on Tukey's post-hoc analysis, $P < 0.05$)

Tang et al. 1993b), as well as decreased root elongation after exposure to a nutrient solution of pH ≥ 6 for one hour (Tang et al. 1992). Tang et al. (1993b) also found decreased root cell elongation in *L. angustifolius* in nutrient solution of pH ≥ 6. Yu and Tang (2000) suggested that the difference in H^+-buffering and Ca^{2+}-exchange capacities in the root apoplast is related to the sensitivity of root growth of *L. angustifolius* and *Pisum sativum* to high pH, and that the greater sensitivity of *L. angustifolius* roots to high pH can be partly explained by a higher H^+ requirement for cell-wall loosening. According to this explanation, the better root growth of *L. pilosus* might be the result of higher H^+-buffering capacity and a high proportion of easily exchangeable Ca^{2+}. There are specific PM H^+-ATPases, as discussed below, that improve the root growth of *L. pilosus* at high pH.

Solution pH and leaf chlorosis

Leaf chlorosis in relation to Fe or Mn deficiency has been observed in *Lupinus* species growing in calcareous

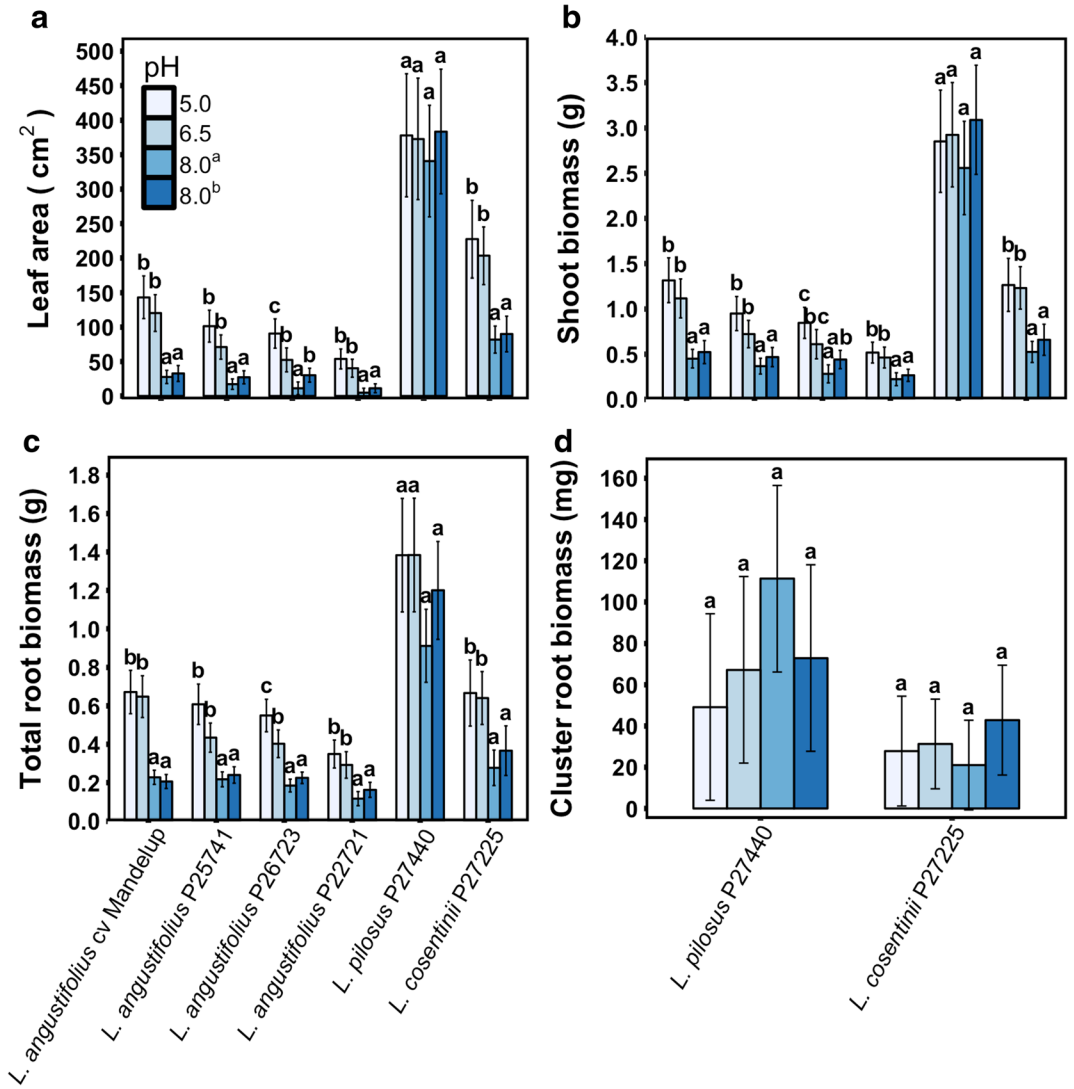


Fig. 8 Leaf area (**a**), shoot (**b**), and total root (**c**) dry biomass of six *Lupinus* genotypes and cluster-root (**d**) dry biomass of two *Lupinus* species when grown at different pH. The pH 5, 6.5 and 8^a were adjusted by KOH and buffered with MES and (0.5 mM each), whereas 8^b was adjusted by $KHCO_3$ without MES or TES added). Error bars represent 95% confidence intervals. Letters show significant differences of different pH within each genotype (based on Tukey's post-hoc analysis, $P < 0.05$)

soils (Brand et al. 2002; George et al. 2012; Moraghan 1991; White and Robson 1989a), in nutrient solution with a high [HCO_3^-] and/or high pH (Bertoni et al. 1992; White and Robson 1990), or in nutrient solution with a low [Fe] (White and Robson 1989b) or [Mn] (Zornoza et al. 2010). In the present study, all *L. angustifolius* genotypes and *L. cosentinii* exhibited leaf chlorosis at pH 8, although the whole leaf [Fe] of these genotypes were similar to those at pH 6.5, and the leaf [Mn] were above the concentration considered adequate for crop species (Kirkby 2012). However, total [Fe] or [Mn] in shoot dry weight are unreliable predictors of deficiency (Brennan 1999; Hannam et al. 1985; Jessop et al. 1990), because Mn is available to plants only as Mn^{2+} and plants predominantly require Fe^{2+}, rather than Fe^{3+} (Broadley et al. 2012; Grillet and Schmidt 2017; Pittman 2005). Increased pH (from 6.5 to 8) in the leaf apoplast restricts Fe^{3+}- or Mn^{4+}- reductase activity (Kosegarten et al. 2004; Mengel 1994; Römheld 2000; Zohlen and Tyler 2000), reducing Fe

and/or Mn availability. In contrast with *L. angustifolius* and *L. cosentinii*, leaf chlorosis of *L. cosentinii* was very light, and we did not observe any chlorosis in *L. pilosus* at pH 8. The increased leaf concentrations of K, together with the greater translocation of K to leaves in *L. cosentinii* and *L. pilosus* may be related to the activity of high pH-activated membrane-bound proton-pumping ATPases, resulting in a more acidic apoplastic pH and improving Fe^{2+} or Mn^{2+} utilisation (Jolley et al. 2004; Tagliavini and Rombolà 2001).

The effect of pH on nutrient uptake and translocation

The net P-uptake capacity of all the *L. angustifolius* genotypes and *L. cosentinii* was less at pH 8, while there was no difference in the net P-uptake capacity of *L. pilosus*. This is probably because lateral root growth of all the *L. angustifolius* genotypes and *L. cosentinii* was inhibited at pH 8, and the root surface area was decreased as well, likely accounting for a lower P-uptake capacity (Niu et al. 2013). The uptake of Fe, Zn, Mn, K and Ca of all the *L. angustifolius* genotypes and *L. cosentinii* was inhibited at pH 8 as well, because of decreased lateral root growth and reduced nutrient solubility in nutrition solution at high [HCO_3^-] and/or high pH (George et al. 2012; Yang et al. 1994). However, we only observed lower uptake of P, Fe and Zn at pH 8 which could be caused by decreased P, Fe and Zn solubility at pH 8 (George et al. 2012; Yang et al. 1994; Table S1).

A high pH in the root apoplast could restrict Fe^{3+}- and/or Mn^{4+}-reductase activity and decrease Fe and/or Mn translocation to leaves, and hence more accumulated in the roots (Kosegarten and Koyro 2001; Mengel 1994; Millaleo et al. 2010; Rengel 2000; Zribi and Gharsalli 2002). This agrees with our results showing that root [Mn] of all the genotypes was generally greater at pH 8 than at pH 5 and 6.5, and the Fe and Mn translocation to leaves of all genotypes was inhibited at high pH. In addition, the root Fe^{3+}-reducing capacity requires the availability of metal ions (e.g., Fe^{2+}, Zn^{2+}, Mn^{2+}) (Romera et al. 1997), while the availability of these ions is relatively low at high pH which might result in decreased root Fe^{3+}-reducing capacity. Interestingly, pH 8.0^a, adjusted by KOH, had a more severe negative effect on the translocation of P, Fe, Zn, Mn and K in *L. angustifolius* than pH 8.0^b, adjusted by $KHCO_3$. This is probably because high HCO_3^- supply enhanced root PEP-carboxylase activity, producing organic acids (Abadía et al. 2002; Miller et al. 1990) to be transported to the leaves (López-Millán et al. 2000) or increase the availability of nutrients to be transported to leaves (Jones 1998).

Low leaf [P] or [Fe] can trigger cluster-root formation, and/or the capacity to release protons and carboxylates in *Lupinus* species, thus increasing the solubility of P and Fe (Dinkelaker et al. 1989; Lambers et al. 2013; Liang and Li 2003; White and Robson 1989b). However, we did not observe an increase in cluster-root formation in *L. pilosus* and *L. cosentinii* from pH 5 to pH 8. A high [HCO_3^-] and/or high pH may have inhibited cluster-root formation or carboxylate release in response to endogenous [P] or [Fe].

The effect of pH on P and Ca allocation

As previously observed (Ding et al. 2018b), P was preferentially allocated to epidermal cells, and Ca to mesophyll cells, especially palisade mesophyll cells. This has been found before in a range of dicots (Conn and Gilliham 2010), but is not typical for all dicots (Hayes et al. 2018; Guilherme Pereira et al. 2018). At higher pH, the [P] in epidermal cells of all genotypes was significantly lower; this agrees with the P-uptake capacity and whole leaf [P] results. The change of [Ca] in the mesophyll cells of all genotypes was complex. The most interesting observation was the [Ca] of *L. angustifolius* P26723 and *L. pilosus*, whose [Ca] in the mesophyll cells at pH 8.0^b were much lower than those at pH 8.0^a. We did not find any increase in [Ca] of the epidermal or bundle sheath cells in *L. angustifolius* P26723 and *L. pilosus* at pH 8.0^b. However, their whole leaf [Ca], should be higher at pH 8.0^a than those at pH 8.0^b according to leaf cellular [Ca], which was very similar at pH 8.0^a and 8.0^b. This means there might be some Ca in the leaf apoplast at pH 8.0^b.

The relationship between nutrient status and photosynthesis

Photosynthesis requires P, Fe and Mn (Broadley et al. 2012; Hawkesford et al. 2012; Mengel and Kirkby 2001), and the rate of photosynthesis of all *L. angustifolius* genotypes and *L. cosentinii* decreased at pH 8, while that of *L. pilosus* was only slightly decreased at pH 8.0^a. The photosynthetic rate of *L. angustifolius* P25741, *L. angustifolius* P22721,

L. pilosus, and *L. cosentinii* at pH 8.0[a] was even slower than that at pH 8.0[b]. In addition, the quantum yield of PSII (*Fv*/*Fm*) of all genotypes were significantly lower at pH 8 than at pH 5 and 6.5, indicating that the PSII activity of all genotypes was reduced at pH 8 (Maxwell and Johnson 2000). We observed P-deficiency and chlorosis symptoms in young leaves of all of the *L. angustifolius* genotypes and *L. cosentinii* at pH 8, and the leaf chlorosis may be related with either Fe- or Mn-deficiency, while we only observed P-deficiency symptoms in *L. pilosus* at pH 8. Therefore, the decreased photosynthetic capacity of all the *L. angustifolius* genotypes was caused by P, Fe or Mn deficiency, or a combination thereof. However, the low A_{max} of *L. pilosus* was consistent with its low leaf [Mn], which is much lower than the critical shoot required for the growth of *L. angustifolius* [Mn] (277 mg kg^{-1}), so the decreased A_{max} of this species was likely due to a decreased leaf [Mn] caused by pH 8.

Similarities and differences between the results in this study and those in other studies

Generally, our findings agree with the natural occurrence of the species; for example, *L. angustifolius* naturally grows on acidic soils, and *L. pilosus* on more alkaline soils (Clements and Cowling 1990). Other studies also showed similar nutrient imbalances at high [HCO_3^-] and/or high pH. For example, Kerley et al. (2001) reported that leaf [P], [Fe], [Mn] and [Zn] of *L. albus*, *L. angustifolius* and *L. pilosus* are lower when plants grow in calcareous soils. In the same study, Kerley et al. (2001) also found that leaf [P] was below the critical concentration in *L. albus*, *L. angustifolius* and *L. pilosus* when grown in calcareous soils. In addition, Fe translocation of *L. albus*, *L. angustifolius* and *L. pilosus* from stems to leaves was negatively affected by high [HCO_3^-] (Kerley et al. 2001). A negative effect of high [HCO_3^-] on leaf [Zn] and [Fe] was also found for other species (Dogar and Van Hai 1980; Fleming et al. 1984; Forno et al. 1975; Gharsalli and Hajji 2002). Our results indicate that pH is the main reason why some *Lupinus* species are sensitive to calcareous soils. Interestingly, comparing effects of pH 8.0[a] and 8.0[b], pH 8.0[a] gave a stronger inhibition of Mn translocation to leaves in all *L. angustifolius* genotypes and *L. cosentinii*. Also, leaf cellular [Ca] of *L. angustifolius* P26723 and *L. pilosus* were lower at pH 8.0[b] than at pH 8.0[a]. This probably reflects increased HCO_3^- assimilation and formation of organic acids in roots at high HCO_3^- supply which resulted in greater Mn translocation to leaves. At the same time, bicarbonate-enhanced Mn^{2+}-translocation would compete with Ca for the plasma-membrane Ca^{2+} channel in *L. angustifolius* P26723 (White 2000).

We found that leaf K concentrations of *L. pilosus* and *L. cosentinii* at pH 8 were higher than those at pH 5 and 6.5 which could be the reason why these two species had no or less leaf chlorosis symptoms. This is probably because K is involved in activating membrane-bound proton-pumping ATPases which resulted in a more acidic apoplastic pH and improved Fe and Mn availability (Barak and Chen 1984; Jolley et al. 2004). We also found that more Ca was translocated to leaves at pH 8 than at pH 5 and 6.5 in these two species which suggests that Ca could be related to the alleviation of negative effects caused by high pH, as Ca could be involved in decreasing K loss through the inhibition of the K outward-rectified channels, thus maintaining K concentrations (Gómez-Pérez et al. 2014; Murata et al. 2000). Further studies are needed to test this.

Conclusions

A high pH inhibited lateral root growth of *L. angustifolius* genotypes and *L. cosentinii*, decreased photosynthetic rates, caused leaf chlorosis and inhibited P uptake. It also inhibited uptake of Fe and Zn in all genotypes and that of Mn, K and Ca in all sensitive species, as well as translocation of P, Fe, Zn, Mn and Ca of most sensitive species from roots to leaves. However, a high pH increased K translocation to leaves in *L. pilosus* and *L. cosentinii* which may be related with the activity of H^+-ATPases and then their relative tolerance or lower sensitivity to pH 8. Bicarbonate decreased the negative effect of pH 8.0 on nutrient translocation to leaves in most *L. angustifolius* genotypes. Decreased growth of all *L. angustifolius* genotypes and *L. cosentinii* was associated with decreased lateral-root growth, photosynthetic rate and P, Fe or Mn deficiencies. High pH did not affect the leaf cell types that accumulated P and Ca, but the leaf cellular [P] decreased at high pH. This knowledge provides critical insights into the calcicole or calcifuge habits of *Lupinus* species and other plants which can be used to guide breeding of calcicole plants to improve their production and use.

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References

Abadía J, López-Millán A-F, Rombolà A, Abadía A (2002) Organic acids and Fe deficiency: a review. Plant Soil 241: 75–86

Barak P, Chen Y (1984) The effect of potassium on iron chlorosis in calcareous soils. J Plant Nutr 7:125–133

Bertoni GM, Pissaloux A, Morard P, Sayag DR (1992) Bicarbonate-pH relationship with iron chlorosis in white lupine. J Plant Nutr 15:1509–1518

Brand JD, Tang C, Rathjen AJ (1999) Adaptation of *Lupinus angustifolius* L. and *L. pilosus* Murr. to calcareous soils. Aust J Agric Res 50:1027–1034

Brand JD, Tang C, Graham RD (2000) The effect of soil moisture on the tolerance of *Lupinus pilosus* genotypes to a calcareous soil. Plant Soil 219:263–271

Brand JD, Tang C, Rathjen AJ (2002) Screening rough-seeded lupins (*Lupinus pilosus* Murr. and *Lupinus atlanticus* Glads.) for tolerance to calcareous soils. Plant Soil 245:261–275

Brennan RF (1999) Lupin grain yields and fertiliser effectiveness are increased by banding manganese below the seed. Aust J Exp Agric 39:595–603

Broadley M, Brown P, Cakmak I, Rengel Z, Zhao F (2012) Function of nutrients: micronutrients. In: Marschner P (ed) Marschner's mineral nutrition of higher plants, 3rd edn. Academic Press, San Diego

Bugbee BG, Salisbury FB (1985) An evaluation of MES (2(N-Morpholino) ethanesulfonic acid) and amberlite IRC-50 as pH buffers for nutrient solution studies. J Plant Nutr 8:567–583

Burnham KP, Anderson DR (2003) Model selection and multimodel inference: a practical information-theoretic approach. Springer Science & Business Media, New York

Clements JC, Cowling WA (1990) The Australian lupin collection - passport data for wild and semi-domesticated accessions introduced into Australia to 1990. Western Australian Department of Agriculture, Perth

Conn S, Gilliham M (2010) Comparative physiology of elemental distributions in plants. Ann Bot 105:1081–1102

Coulombe BA, Chaney RL, Wiebold WJ (1984a) Bicarbonate directly induces iron chlorosis in susceptible soybean cultivars1. Soil Sci Soc Am J 48:1297–1301

Coulombe BA, Chaney RL, Wiebold WJ (1984b) Use of bicarbonate in screening soybeans for resistance to iron chlorosis. J Plant Nutr 7:411–425

De Silva BLT (1934) The distribution of "calcicole" and "calcifuge" species in relation to the content of the soil in calcium carbonate and exchangeable calcium, and to soil reaction. J Ecol 22:532–553

De Silva DLR, Ruiz LP, Atkinson CJ, Mansfield TA (1994) Physiological disturbances caused by high rhizospheric calcium in the calcifuge *Lupinus luteus*. J Exp Bot 45:585–590

Ding W, Clode PL, Clements JC, Lambers H (2018a) Effects of calcium and its interaction with phosphorus on the nutrient status and growth of three *Lupinus* species. Physiol Plant 163:386–398

Ding W, Clode PL, Clements JC, Lambers H (2018b) Sensitivity of different *Lupinus* species to calcium under a low phosphorus supply. Plant Cell Environ 41:1512–1523

Dinkelaker B, Römheld V, Marschner H (1989) Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell Environ 12:285–292

Dinkelaker B, Hengeler C, Marschner H (1995) Distribution and function of Proteoid roots and other root clusters. Bot Acta 108:183–200

Dogar MA, Van Hai T (1980) Effect of P, N and HCO_3^- levels in the nutrient solution on rate of Zn absorption by rice roots and Zn content in plants. Z Pflanzenphysiol 98:203–212

Fleming AL, Chaney RL, Coulombe BA (1984) Bicarbonate inhibits Fe-stress response and Fe uptake-translocation of chlorosis-susceptible soybean cultivars. J Plant Nutr 7:699–714

Forno DA, Yoshida S, Asher CJ (1975) Zinc deficiency in rice. Plant Soil 42:537–550

Fox J (2003) Effect displays in R for generalised linear models. J Stat Softw 8:1–27

George E, Horst WJ, Neumann E (2012) Adaptation of plants to adverse chemical soil conditions. In: Marschner P (ed) Marschner's mineral nutrition of higher plants, 3rd edn. Academic Press, San Diego

Gharsalli M, Hajji M (2002) Comparison of physiological responses of peach and almond seedlings to iron deficiency. J Plant Nutr 25:1139–1154

Gladstones J (1974) Lupins of the Mediterranean region and Africa. Technical Bulletins. Department of Agriculture and Food, Perth

Gómez-Pérez L, Valdez-Aguilar LA, Sandoval-Rangel A, Benavides-Mendoza A, Mendoza-Villarreal R, Castillo-González AM (2014) Calcium ameliorates the tolerance of lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] to alkalinity in irrigation water. HortScience 49:807–811

Grillet L, Schmidt W (2017) The multiple facets of root iron reduction. J Exp Bot 68:5021–5027

Guilherme Pereira C, Clode PL, Oliveira RS, Lambers HC (2018) Eudicots from severely phosphorus-impoverished

environments preferentially allocate phosphorus to their mesophyll. New Phytol 218:959–973
Hannam RJ, Graham RD, Riggs JL (1985) Diagnosis and prognosis of manganese deficiency in *Lupinus angustifolius* L. Aust J Agric Res 36:765–777
Hawkesford M, Horst W, Kichey T, Lambers H, Schjoerring J, Møller IS, White P (2012) Functions of macronutrients. In: Marschner P (ed) Marschner's mineral nutrition of higher plants, 3rd edn. Academic Press, San Diego
Hayes PE, Clode PL, Oliveira RS, Lambers H (2018) Proteaceae from phosphorus-impoverished habitats preferentially allocate phosphorus to photosynthetic cells: an adaptation improving phosphorus-use efficiency. Plant Cell Environ 41: 605–619
Hsieh E-J, Waters BM (2016) Alkaline stress and iron deficiency regulate iron uptake and riboflavin synthesis gene expression differently in root and leaf tissue: implications for iron deficiency chlorosis. J Exp Bot 67:5671–5685
Huang CX, Canny MJ, Oates K, McCully ME (1994) Planing frozen hydrated plant specimens for SEM observation and EDX microanalysis. Microsc Res Tech 28:67–74
Impa SM, Morete MJ, Ismail AM, Schulin R, Johnson-Beebout SE (2013) Zn uptake, translocation and grain Zn loading in rice (*Oryza sativa* L.) genotypes selected for Zn deficiency tolerance and high grain Zn. J Exp Bot 64:2739–2751
Jessop RS, Roth G, Sale P (1990) Effects of increased levels of soil $CaCO_3$ on lupin (*Lupinus angustifolius*) growth and nutrition. Soil Res 28:955–962
Jolley VD, Hansen NC, Shiffler AK (2004) Nutritional and management related interactions with iron-deficiency stress response mechanisms. Soil Sci Plant Nutr 50:973–981
Jones HG (1985) Partitioning stomatal and non-stomatal limitations to photosynthesis. Plant Cell Environ 8:95–104
Jones DL (1998) Organic acids in the rhizosphere – a critical review. Plant Soil 205:25–44
Karimi HR, Tari FE (2017) Effects of $NaHCO_3$ on photosynthetic characteristics, and iron and sodium transfer in pomegranate. J Plant Nutr 40:11–22
Kerley SJ (2000) The effect of soil liming on shoot development, root growth, and cluster root activity of white lupin. Biol Fertil Soils 32:94–101
Kerley SJ, Huyghe C (2002) Stress-induced changes in the root architecture of white lupin (*Lupinus albus*) in response to pH, bicarbonate, and calcium in liquid culture. Ann Appl Biol 141:171–181
Kerley SJ, Shield IF, Huyghe C (2001) Specific and genotypic variation in the nutrient content of lupin species in soils of neutral and alkaline pH. Aust J Agric Res 52:93–102
Kirkby E (2012) Introduction, definition and classification of nutrients. In: Marschner P (ed) Marschner's mineral nutrition of higher plants, 3rd edn. Academic press, San Diego
Kosegarten H, Koyro H-W (2001) Apoplastic accumulation of iron in the epidermis of maize (*Zea mays*) roots grown in calcareous soil. Physiol Plant 113:515–522
Kosegarten HU, Hoffmann B, Mengel K (1999) Apoplastic pH and Fe^{3+} reduction in intact sunflower leaves. Plant Physiol 121:1069–1079
Kosegarten H, Hoffmann B, Rroco E, Grolig F, Glüsenkamp K-H, Mengel K (2004) Apoplastic pH and FeIII reduction in young sunflower (*Helianthus annuus*) roots. Physiol Plant 122:95–106
Lambers H, Chapin IIIFS, Pons TL (2008) Mineral nutrition. Plant physiological ecology, 2nd edn. Springer, New York
Lambers H, Clements JC, Nelson MN (2013) How a phosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupins (*Lupinus*, fabaceae). Am J Bot 100:263–288
Lee JA, Woolhouse HW (1969) A comparative study of bicarbonate inhibition of root growth in calcicole and calcifuge grasses. New Phytol 68:1–11
Liang R, Li C (2003) Differences in cluster-root formation and carboxylate exudation in *Lupinus albus* L. under different nutrient deficiencies. Plant Soil 248:221–227
Loneragan J, Snowball K (1969) Calcium requirements of plants. Aust J Agric Res 20:465–478
López-Millán AF, Fn M, Abadía A, Abadía J (2000) Effects of iron deficiency on the composition of the leaf apoplastic fluid and xylem sap in sugar beet. Implications for iron and carbon transport. Plant Physiol 124:873–884
Lucena C, Romera FJ, Rojas CL, García MJ, Alcántara E, Pérez-Vicente R (2007) Bicarbonate blocks the expression of several genes involved in the physiological responses to Fe deficiency of strategy I plants. Funct Plant Biol 34:1002–1009
Marshall AT (2017) Quantitative x-ray microanalysis of model biological samples in the SEM using remote standards and the XPP analytical model. J Microsc 266:231–238
Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. J Exp Bot 51:659–668
McCully ME, Canny MJ, Huang CX, Miller C, Brink F (2010) Cryo-scanning electron microscopy (CSEM) in the advancement of functional plant biology: energy dispersive X-ray microanalysis (CEDX) applications. Funct Plant Biol 37: 1011–1040
Mengel K (1994) Iron availability in plant tissues-iron chlorosis on calcareous soils. Plant Soil 165:275–283
Mengel K, Kirkby EA (2001) Principles of plant nutrition. Kluwer Academic Publishers, Dordrecht
Mengel K, Breininger MT, Bübl W (1984) Bicarbonate, the most important factor inducing iron chlorosis in vine grapes on calcareous soil. Plant Soil 81:333–344
Mengel K, Planker R, Hoffmann B (1994) Relationship between leaf apoplast pH and iron chlorosis of sunflower (*Helianthus annuus* L.). J Plant Nutr 17:1053–1065
Millaleo R, Reyes-Díaz M, Ivanov AG, Mora ML, Alberdi M (2010) Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. J Soil Sci Plant Nutr 10:470–481
Miller GW, Shigematsu A, Welkie GW, Motoji N, Szlek M (1990) Potassium effect on iron stress in tomato. J Plant Nutr 13: 1355–1370
Moraghan JT (1991) The growth of white lupine on a calciaquoll. Soil Sci Soc Am J 55:1353–1357
Motomizu S, Wakimoto T, Tôei K (1983) Spectrophotometric determination of phosphate in river waters with molybdate and malachite green. Analyst 108:361–367
Murata Y, Katsura S, Obi I, Kakutani T (2000) Alterations in Ca^{2+}-binding on plasma membrane after adaptation to salt stress of tobacco cells in suspension. Plant Cell Physiol 41:1286–1292
Neumann G, Römheld V (2012) Rhizosphere chemistry in relation to plant nutrition. In: Marschner P (ed) Marschner's mineral

nutrition of higher plants, 3rd edn. Academic Press, San Diego

Neumann G, Massonneau A, Langlade N, Dinkelaker B, Hengeler C, Römheld V, Martinoia E (2000) Physiological aspects of cluster root function and development in phosphorus-deficient white lupin (*Lupinus albus* L.). Ann Bot 85:909–919

Nichols DG, Beardsell DV (1981) Interactions of calcium, nitrogen and potassium with phosphorus on the symptoms of toxicity in *Grevillea* cv. 'Poorinda Firebird'. Plant Soil 61: 437–445

Niu YF, Chai RS, Jin GL, Wang H, Tang CX, Zhang YS (2013) Responses of root architecture development to low phosphorus availability: a review. Ann Bot 112:391–408

Parker DR, Norvell WA, Sparks DL (1999) Advances in solution culture methods for plant mineral nutrition research. Adv Agron 65:151–213

Peiter E, Yan F, Schubert S (2001) Lime-induced growth depression in *Lupinus* species: are soil pH and bicarbonate involved? J Plant Nutr Soil Sci 164:165–172

Pittman JK (2005) Managing the manganese: molecular mechanisms of manganese transport and homeostasis. New Phytol 167:733–742

R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Rengel Z (2000) Manganese uptake and transport in plants. In: Astrid S, Helmut S (eds) Metal ions in biological systems. Marcel Dekker, New York

Romera FJ, Alcántara E, de la Guardia MD (1991) Characterization of the tolerance to iron chlorosis in different peach rootstocks grown in nutrient solution. Plant Soil 130: 121–125

Romera FJ, Alcántara E, de la Guardia MD (1992) Effects of bicarbonate, phosphate and high pH on the reducing capacity of Fe-deficient sunflower and cucumber plants. J Plant Nutr 15:1519–1530

Romera FJ, Alcántara E, MDd l G (1997) Influence of bicarbonate and metal ions on the development of root Fe(III) reducing capacity by Fe-deficient cucumber (*Cucumis sativus*) plants. Physiol Plant 101:143–148

Römheld V (2000) The chlorosis paradox: Fe inactivation as a secondary event in chlorotic leaves of grapevine. J Plant Nutr 23:1629–1643

Rutland RB, Bukovac MJ (1971) The effect of calcium bicarbonate on iron absorption and distribution by *Chrysanthemum morifolium*, (Ram.). Plant Soil 35:225–236

Shaff JE, Schultz BA, Craft EJ, Clark RT, Kochian LV (2010) GEOCHEM-EZ: a chemical speciation program with greater power and flexibility. Plant Soil 330:207–214

Shane MW, Lambers H (2005) Cluster roots: a curiosity in context. Plant Soil 274:101–125

Snowball K, Robson AD (1986) Symptoms of nutrient deficiencies: Lupins. Department of Soil Science and Plant Nutrition, Institute of Agriculture, University of Western Australia

Tagliavini M, Rombolà AD (2001) Iron deficiency and chlorosis in orchard and vineyard ecosystems. Eur J Agron 15:71–92

Tang C, Robson AD (1993) pH above 6.0 reduces nodulation in *Lupinus* species. Plant Soil 152:269–276

Tang C, Robson AD (1995) Nodulation failure is important in the poor growth of two lupin species on an alkaline soil. Anim Prod Sci 35:87–91

Tang C, Thomson BD (1996) Effects of solution pH and bicarbonate on the growth and nodulation of a range of grain legume species. Plant Soil 186:321–330

Tang C, Longnecker NE, Thomson CJ, Greenway H, Robson AD (1992) Lupin (*Lupinus angustifolius* L.) and pea (*Pisum sativum* L.) roots differ in their sensitivity to pH above 6.0. J Plant Physiol 140:715–719

Tang C, Kuo J, Longnecker NE, Thomson CJ, Robson AD (1993a) High pH causes disintegration of the root surface in *Lupinus angustifolius* L. Ann Bot 71:201–207

Tang C, Robson AD, Longnecker NE, Greenway H (1993b) Physiological responses of lupin roots to high pH. Plant Soil 155:509–512

Tang C, Robson AD, Longnecker NE, Buirchell BJ (1995) The growth of *Lupinus* species on alkaline soils. Crop Pasture Sci 46:255–268

Tang C, Adams H, Longnecker NE, Robson AD (1996) A method to identify lupin species tolerant of alkaline soils. Anim Prod Sci 36:595–601

Tansley AG (1917) On competition between *Galium saxatile* L. (*G. hercynicum* Weig.) and *Galium sylvestre* Poll. (*G. asperum* Schreb.) on different types of soil. J Ecol 5: 173–179

Tyler G, Ström L (1995) Differing organic acid exudation pattern explains calcifuge and acidifuge behaviour of plants. Ann Bot 75:75–78

Waters BM, Troupe GC (2012) Natural variation in iron use efficiency and mineral remobilization in cucumber (*Cucumis sativus*). Plant Soil 352:185–197

White PF (1990) Soil and plant factors relating to the poor growth of *Lupinus* species on fine-textured, alkaline soils - a review. Aust J Agric Res 41:871–890

White PJ (2000) Calcium channels in higher plants. Biochim Biophys Acta 1465:171–189

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182:49–84

White PF, Robson AD (1989a) Poor soil aeration or excess soil $CaCO_3$ induces Fe deficiency in lupins. Aust J Agric Res 40: 75–84

White PF, Robson AD (1989b) Rhizosphere acidification and Fe^{3+} reduction in lupins and peas: Iron deficiency in lupins is not due to a poor ability to reduce Fe^{3+}. Plant Soil 119:163–175

White PF, Robson AD (1990) Response of lupins (*Lupinus angustifolius* L.) and peas (*Pisum sativum* L.) to Fe deficiency induced by low concentrations of Fe in solution or by addition of HCO_3. Plant Soil 125:39–47

Yang X, Römheld V, Marschner H (1994) Effect of bicarbonate on root growth and accumulation of organic acids in Zn-inefficient and Zn-efficient rice cultivars (*Oryza sativa* L.). Plant Soil 164:1–7

Yu Q, Tang C (2000) Lupin and pea differ in root cell wall buffering capacity and fractionation of apoplastic calcium. J Plant Nutr 23:529–539

Yue Ao T, Chaney RL, Korcak RF, Fan F, Faust M (1987) Influence of soil moisture level on apple iron chlorosis development in a calcareous soil. Plant Soil 104:85–92

Zohlen A, Tyler G (2000) Immobilization of tissue iron on calcareous soil: differences between calcicole and calcifuge plants. Oikos 89:95–106

Zornoza P, Sánchez-Pardo B, Carpena RO (2010) Interaction and accumulation of manganese and cadmium in the manganese accumulator *Lupinus albus*. J Plant Physiol 167:1027–1032

Zribi K, Gharsalli M (2002) Effect of bicarbonate on growth and iron nutrition of pea. J Plant Nutr 25:2143–2149

Zuur AF, Leno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Spring Science and Business Media, New York