Is occluded phosphate plant-available?

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Abstract

Background: The phosphate concentration of the soil solution is generally low, allowing sufficient plant nutrition only for a few days. Therefore, supply from various fractions of bound phosphate is essential to meet plant demand. It is known that plants have developed strategies to acquire phosphorus (P) from phosphates adsorbed on clay minerals or oxides, from organically bound phosphates, and from calcium phosphates. However, it is generally assumed that occluded phosphate is not plant-available.
Results: In a pot experiment, two plant species, namely maize (*Zea mays* L.) and white lupin (*Lupinus albus* L.), differing in acquisition efficiency, were used to investigate whether Al oxide-occluded and Fe oxide-occluded phosphates can be acquired. Artificially prepared Al oxide-occluded phosphate or Fe oxide-occluded phosphate, respectively, was added to a subsoil low in available phosphates. It is shown that both plant species were not able to acquire P from Al oxide-occluded phosphate. Also, maize was incapable of using Fe oxide-occluded phosphate. In contrast, white lupin took up significant amounts of P from Fe oxide-occluded phosphate.
Conclusion: It is concluded that the strategy to form cluster roots together with their reducing power may allow white lupin to destabilize Fe oxides that occlude phosphates and to mine the soil for this additional phosphate fraction.

Key words: iron reduction / *Lupinus albus* / nutrient acquisition / phosphate fractions / *Zea mays*

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1 Introduction

The phosphorus (P) bioavailability in soils is low (*Filipelli*, 2008). Due to binding of phosphate to organic and inorganic soil particles, the concentration of P in the soil solution is generally low (*Pierre* and *Parker*, 1927; *Hinsinger*, 2001; *Smith* et al., 2003). Phosphate in the soil solution is sufficient to feed rapidly growing plants for only a few days (*Mengel* and *Kirkby*, 2001). Thus, various fractions of bound phosphates must contribute to plant P nutrition. These comprise phosphates adsorbed to clay minerals and Al and Fe (hydr)oxides (*Hinsinger* et al., 2005; *Wang* et al., 2013), organically bound phosphates (*Asmar* et al., 1995; *Steffens* et al., 2010), calcium phosphates (*Aguilar* and *van Diest*, 1981), iron phosphates (*Ae* et al., 1990), and aluminum phosphates (*Lindsay* et al., 1989). Depending on soil type and soil pH the abundance of these fractions varies to a large degree and, therefore, the contribution to plant nutrition is soil-specific (*Gerke*, 2015; *Hallama* et al., 2019).

In addition, different plant species have developed specific strategies to cope with sparingly soluble P. Acquisition of P requires three steps, *i.e.*, P mobilization, P transport to the root surface (predominantly by diffusion), and uptake by root cells (*via* proton/phosphate cotransporters; *Smith* et al., 2003). All three processes may be optimized to improve P acquisition. The strategy of P mobilization depends on the kind of phosphate binding involved. Adsorbed phosphate can be made available by ligand exchange with hydroxyls (*Wang* et al., 2013) which are released by root cells during physiologically alkaline nutrition (*Schubert* and *Yan*, 1997), a process that may be of particular importance in acid soils. For ligand exchange of adsorbed phosphate plants may also release organic anions such as malate, oxalate or citrate (*Gahoonia* et al., 2000; *Hinsinger*, 2001; *Aziz* et al., 2011; *Gerke*, 2015) and mucilage (*Gaume* et al., 2000). Organically bound phosphate may be hydrolytically made available by activity of phosphatase (*Asmar* et al., 1995; *Wasaki* et al., 2003; *Steffens* et al., 2010). The capability of mobilizing organically bound phosphate depends on the plant nutritional status (*Beißner* and *Römer*, 1999). Large differences in mobilization of organically bound phosphate have been demonstrated for various plant species with rape being a particularly efficient species and potato a distinctly inefficient species when dependent predominantly on the root system (and not on mycorrhization) and when the microbial activity of the soil is low (*Steffens* et al., 2010; *Wening*, 2016). Calcium phosphates may be mobilized by rhizosphere acidification and also for this P fraction it was demonstrated that rape is efficient due to high calcium uptake and rhizosphere acidification at the root tip.

In most cases, transport of phosphate to the root surface cannot directly be influenced by the plant. However, because phosphate is predominantly transported *via* diffusion (*Mengel*

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and *Kirkby*, 2001), the distance of phosphate from the root surface is decisive. Therefore, a large root surface area decreases the mean distance between phosphate and root surface (*Gerke*, 2015). In this respect grasses appear to be superior to many dicots (*Schubert* and *Mengel*, 1989; *Hallama* et al., 2019). The plasmalemma transporters for phosphate uptake seem to be extremely efficient in decreasing the phosphate concentration at the root surface and therefore seem not to limit P uptake from very low concentrations in the rhizosphere (*Hallama* et al., 2019).

Despite the various strategies of plants to acquire sparingly soluble phosphates, to our knowledge up to now no strategy has been reported that allows plants to acquire occluded phosphates. The latter are formed in acid soils when phosphates are surrounded by Al or Fe oxides that make the phosphates unavailable, a process that contributes to phosphate ageing (*Parfitt* et al., 1975; *Parfitt*, 1979). The aim of the present study was therefore to test two plant species for their ability to acquire P from Al oxide-occluded phosphates and Fe oxide-occluded phosphates. For this purpose, we prepared Al oxide-occluded phosphates and Fe oxide-occluded phosphates and applied them to a soil low in available P forms. We used a subsoil low in microbial activity to avoid P mobilization from these fractions by microorganisms. As test plant we chose maize (*Zea mays* L.) which is regarded as relatively inefficient although it may release hydroxyls during alkaline nutrition (*Schubert* and *Yan*, 1997), thus mobilizing adsorbed phosphate under low-pH conditions. Maize may also mobilize organically bound phosphate (*Steffens* et al., 2010).

The second test plant species was white lupin (*Lupinus albus* L.) which is regarded as very efficient in mobilizing the various phosphate fractions (*Gardner* et al., 1982; *Dinkelaker* et al., 1995; *Neumann* and *Martinoia*, 2002; *Lambers* et al., 2006). It forms cluster roots (also called proteoid roots) that are secondary lateral roots of determinate growth. They form a rhizospheric micro-compartment that allows efficiently changing the chemical composition by various molecular mechanisms (*Gardner* et al., 1982; *Lambers* et al., 2003; *Lamont*, 2003). Proton extrusion by H^+-ATPase activity decreases the pH allowing the solubilization of calcium phosphates (*Neumann* et al., 2000; *Yan* et al., 2002). Anion channels release organic anions such as citrate for ligand exchange of adsorbed phosphates (*Neumann* et al., 2000; *Zhu* et al., 2005). Activity of acid phosphatase favored by proton release makes organically bound phosphates available (*Wasaki* et al., 2003). In addition, the release of phenols may inhibit bacterial phosphatase degradation and has reducing capacity as has the membrane-bound reductase that may reduce Fe^{III} to Fe^{II} and may improve iron nutrition (*Neumann* et al., 2000; *Marschner* and *Römheld*, 1994; *Dinkelaker* et al., 1995; *Neumann* and *Martinoia*, 2002). We speculate that the latter process might also destabilize Fe oxides occluding phosphates thus making them available. In contrast, it may be anticipated that Al oxides cannot be destabilized because Al cannot be reduced. Therefore, the aim of our study was to test the hypothesis that white lupin, in contrast to maize, is able to acquire P from Fe oxide-occluded phosphates but is not able to acquire P from Al oxide-occluded phosphates.

2 Material and methods

2.1 Preparation of occluded phosphates

Occluded phosphates were prepared according to the method for the synthesis of goethite (Fe oxide) and gibbsite (Al oxide) (*Schwertmann* and *Cornell*, 1991). For the preparation of Fe oxide-occluded phosphate 100 mL of 1 M $Fe(NO_3)_3$ solution were filled into a 2-L polyethylene flask; then 180 mL of 5 M KOH solution were added under rapid stirring. Water and 200 mL 0.1 M KH_2PO_4 solution were added to fill up to 2 L and the flask was kept at 70°C for 60 h. The suspension was then washed with deionized water by centrifugation (3840 *g*) for 5 min and, then, dried at 40°C and ground (≤ 1 mm). Then, this ground material was washed sequentially with different extractants (according to the Chang and Jackson method of P fractionation with modifications by others) to remove the phosphate fractions except the occluded phosphate (*Chang* and *Jackson*, 1957; *Fife*, 1959; *Williams* et al., 1967; *Hartikainen*, 1979; *Bowman*, 1989). The Al oxide-occluded phosphate was prepared in the same way as described for Fe oxide-occluded phosphate using $Al(NO_3)_3$ solution.

2.2 Pot culture experiment

The soil used was a Luvisol subsoil that was sieved (< 2 mm) and that is characterized by the physicochemical properties shown in Tab. 1. This soil from Kleinlinden near Giessen was chosen because of its low content of the various plant-available P fractions and its low microbial activity. Prior to sowing the following nutrients were applied to the soil (mg kg^{-1}): 200 N as NH_4NO_3, 250 K as KCl, 50 Mg as $MgSO_4$, 5 Cu as $CuSO_4$, 20 Mn as $MnSO_4$,10 Zn as $ZnSO_4$, 1 B as H_3BO_3, and 0.2 Mo as $(NH_4)_6Mo_7O_{24}$. Maize (*Zea mays* L. cv. Amadeo) and white lupin (*Lupinus albus* L. cv. Amiga) were grown in pots (12.7 mm × 9.8 mm × 9.8 mm; one plant kg^{-1} soil) with four replicates. There were four P treatments: Control (without P application), 10 mg P as Al oxide-occluded P, 10 mg P as Fe oxide-occluded P, and 10 mg P as $Ca(H_2PO_4)_2$. Plants were cultivated in a light/dark cycle of 16 h/8 h with 25°C/18°C. The light intensity was 500 μmol m^{-2} s^{-1} provided by HQI-T 400 W/D q968 lamps (Osram Powerstar, Germany). The water content during the 35 d period was maintained at 60% water-holding capacity.

2.3 Plant analysis

At harvest, plant roots were separated from soil by gently shaking and carefully washing the roots with deionized water. The soil was then analyzed for Al oxide-occluded phosphate and Fe-occluded phosphate. Roots were carefully washed with deionized water. After drying to constant weight at 105°C, a 0.5 g aliquot sample of whole plants was dry-ashed in a crucible at 520°C in a muffle furnace overnight. Then 2 mL double-deionized water and 5 mL 5 M HNO_3 were added and the solution was constantly heated and filtered (white band filter). The P concentration was measured with a spectrophotometer (PM 7, Zeiss) at 450 nm (*Allen* et al., 1974).

Table 1: Physicochemical properties of the Luvisol subsoil used for plant cultivation.

Parameter	Soil
pH (0.01 M $CaCl_2$)	7.2
Total C (mg kg^{-1})	2800
Total N (mg kg^{-1})	200
Total S (mg kg^{-1})	200
CAL-P (mg kg^{-1})	5.94
Fe oxide-adsorbed (P mg kg^{-1})[a]	5.21
Al oxide-adsorbed (P mg kg^{-1})[a]	2.72
Fe oxide-occluded (P mg kg^{-1})[a]	0.94
Al oxide-occluded (P mg kg^{-1})[a]	not detectable
CAL-K (mg kg^{-1})	38.81
Exch. Mg (mg kg^{-1})	166.0
DTPA Cu, (mg kg^{-1})	0.60
DTPA Mn, (mg kg^{-1})	11.03
DTPA Fe, (mg kg^{-1})	34.65
Oxalate Fe (g kg^{-1})	1.42
Dithionite Fe (g kg^{-1})	5.92
Oxalate Al (g kg^{-1})	1.26
Dithionite Al (g kg^{-1})	1.27
CEC (cmol kg^{-1})	9.80
Sand (g kg^{-1})	479
Silt (g kg^{-1})	345
Clay (g kg^{-1})	176
Water-holding capacity (%)	30.0

[a]Phosphate fractionation according to *Chang* and *Jackson* (1957).

2.4 Soil analyses

The pH was determined in 10 g soil (≤ 2 mm) suspended in 25 mL of 0.01 M $CaCl_2$ using a glass electrode connected to a pH meter (Schott CG 805). CAL-extractable P was quantified according to the method of *Schüller* (1969). Soil P fractionation was carried out using the sequential extraction method of *Chang* and *Jackson* (1957). Finely ground (≤ 1 mm) 5 g soil were filled into a centrifuge flask and extracted with 50 mL of 1 M NH_4Cl for 30 min with constant shaking. After centrifugation (3840 *g* for 5 min), the precipitate was washed twice with 25 mL of 10 M NaCl solution and twice with double-distilled water, while the supernatant (which had water-soluble P) was discarded. The precipitate was treated with 50 mL of neutral 0.5 M NH_4F shaking for 1 h. After centrifugation (3840 *g* for 5 min), the precipitate was washed twice with 25 mL of 10 M NaCl solution and twice with double-distilled water while the supernatant (which had AL-P) was discarded. The precipitate was treated with 50 mL of 0.1 M NaOH shaking for 17 h. After centrifugation (3840 *g* for 5 min), the precipitate was washed twice with 25 mL of 10 M NaCl solution and twice with double-distilled water while the supernatant (which had Fe-P) was discarded. The precipitate was treated with 40 mL of 0.3 M sodium citrate and 1 g sodium dithionite ($Na_2S_2O_4$). The suspension was heated in a water bath at 90°C for 15 min with constant shaking. After centrifugation (3840 *g* for 5 min), the precipitate was washed twice with 25 mL of 10 M NaCl solution and twice with double-distilled water while Fe oxide-occluded P was determined in the supernatant using the molybdate blue method (*Murphy* and *Riley*, 1962). Amorphous Fe and Al were extracted with oxalate solution: two grams of soil (≤ 2 mm) were filled into a bottle, 100 mL of oxalate solution were added, and the suspension was shaken in a dark room for 1 h. After filtration, Fe (248.3 nm) and Al (309.3 nm) were determined using atomic absorption spectrophotometry (AAS, Varian Spectra AA 220FS; *McKeague* and *Day*, 1966).

Amorphous and crystalline Fe and Al were extracted with sodium dithionite. Two grams of soil were filled into a 100-mL centrifuge bottle and 40 mL of 0.3 M Na-citrate and 10 mL of 1 M $NaHCO_3$ were added. The suspension was heated at 80°C in a water bath with rapid mixing. One gram of solid sodium dithionite was added, followed by further heating for 5 min. After centrifugation (3840 *g* for 5 min) and filtration, the supernatant was used to determine Fe and Al as described above.

2.5 Statistics

Data are reported as means ± standard errors (SE). The statistical package Sigma Plot 11 was used to test for significant differences among treatments at *P = 5%. Analysis of variance (ANOVA) and Fisher's LSD test were applied to compare treatment means.

3 Results

In comparison to the control, maize dry mass was significantly increased by application of $Ca(H_2PO_4)_2$ but not by occluded phosphate (Fig. 1A). The response of white lupin was different: not only $Ca(H_2PO_4)_2$ but also Fe oxide-occluded phosphate significantly increased dry mass production, though to a lesser extent (Fig. 1B).

In the control treatment without P application, maize took up slightly more P (6.72 mg P) than indicated as available P by the CAL method (5.94 mg P; Tab. 1). Whereas P uptake by maize was doubled by $Ca(H_2PO_4)_2$ application, no significant effect was found for Al oxide-occluded P or Fe oxide-occluded P (Fig. 2A). In contrast, P uptake by white lupin was almost doubled by application of Fe oxide-occluded P and further increased in the treatment with $Ca(H_2PO_4)_2$. In the control treatment, in contrast to maize, white lupin did not take up all the available P indicated by CAL (2.76 *vs.* 5.94 mg P; Fig. 2B, Tab. 1).

Although there was almost 1 mg kg^{-1} Fe oxide-occluded P in the untreated soil (Tab. 1), the application of 10 mg kg^{-1} of Fe

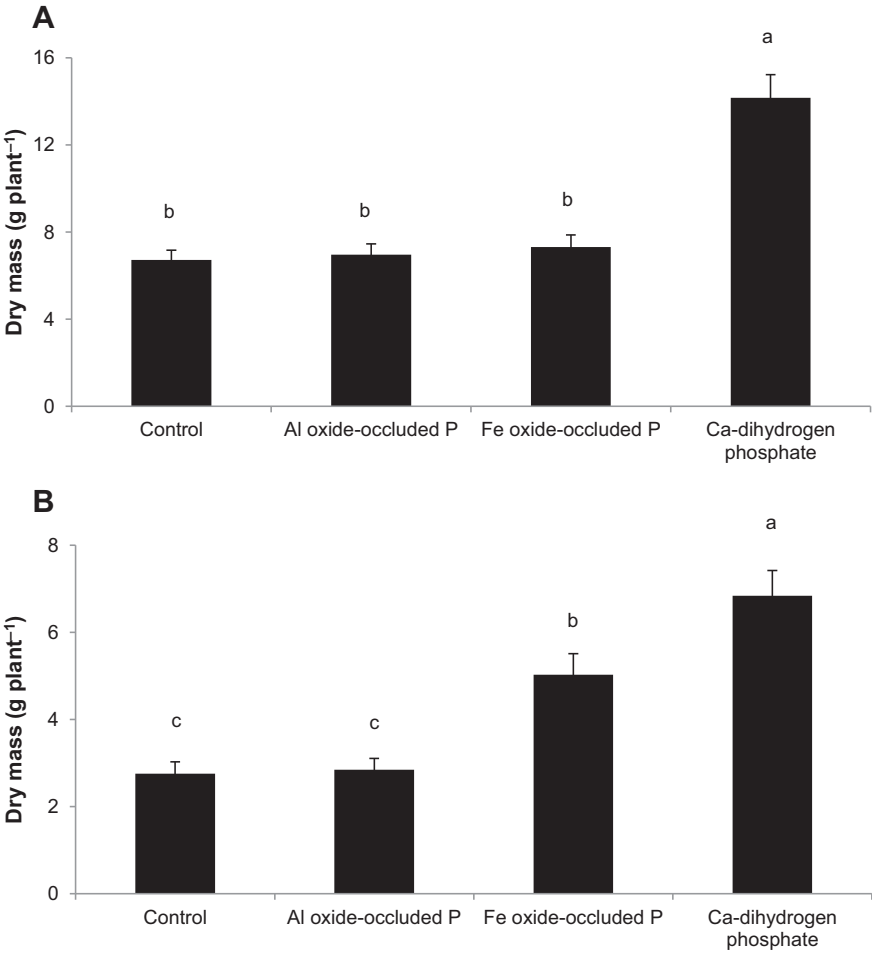

Figure 1: Effect of various phosphate sources on the dry masses of maize (A) and white lupin (B) after 35 d cultivation in a Luvisol subsoil (one plant kg^{-1}). In comparison to a control treatment (without P application), 10 mg P kg^{-1} soil were added as Al oxide-occluded P, Fe oxide-occluded P, or $Ca(H_2PO_4)_2$. Values are means of four replicates ± SE. Columns with different letters indicate significant differences at *P = 5%.

oxide-occluded P did not add up to the expected value of 11 mg P kg^{-1} soil in any of the treatments (Fig. 3). After harvest of maize, neither a significant decrease of Al oxide-occluded P nor a significant decrease of Fe oxide-occluded P was found in the soil indicating that maize was not capable of acquiring occluded phosphate (Fig. 3A). In contrast to maize, a significantly lower value of Fe oxide-occluded P was found in the soil after harvest of white lupin (Fig. 3B). From the added Fe-occluded P white lupin took up 21% (not shown).

4 Discussion

The aim of our study was to test whether plants can acquire occluded phosphate. For this purpose we used a subsoil with low microbiological activity to avoid mobilization of occluded phosphate by microorganisms and with low available phosphate to minimize P uptake from other fractions (Tab. 1). As test plants maize (a putatively inefficient plant) and white lupin (with mechanisms for phosphate mobilization) were employed. Al oxide-occluded phosphate and Fe oxide-occluded phosphate were applied and compared to a soluble phosphate form, namely $Ca(H_2PO_4)_2$. Whereas the addition of $Ca(H_2PO_4)_2$ significantly increased the dry mass of maize and white lupin, Al oxide-occluded phosphate showed no effect (Fig. 1). This may be explained in terms of increased P uptake due to $Ca(H_2PO_4)_2$ application and lack of P-uptake enhancement in the case of Al oxide-occluded phosphate, respectively (Fig. 2). This is in agreement with the general view that Al oxide-occluded phosphate is not plant-available (*Mengel* and *Kirkby*, 2001).

Phosphorus uptake of maize from both the oxide-occluded phosphate treatments was not increased relative to the untreated control (Fig. 2) although maize showed P deficiency (2.95 mg P kg^{-1} dry mass). Thus, if maize had any inducible mechanism to mobilize Fe oxide-occluded phosphate, this mechanism should have become apparent under the experimental conditions. Nitrogen was given as NH_4NO_3; the uptake of nitrate led to alkaline conditions. However, although nitrification of the ammonium may have acidified the bulk soil, transport to the root surface and uptake of nitrate may have contributed to alkaline conditions in the rhizosphere (*Schubert* and *Yan*, 1997) under the soil-pH conditions of 7.2 (Tab. 1). A mobilizing effect from adsorbed fractions due to ligand exchange by the release of hydroxyls (*Wang* et al., 2013) might be conceivable but did not enhance P uptake from occluded fractions added to the soil (Fig. 2A). This reveals that the preparation of occluded fractions did not contain significant amounts of adsorbed phosphate and was thus suitable for the experiment. Also, intensive rooting as occurs in a small pot apparently was not an advantage in mobilizing Fe oxide-occluded phosphate. Although iron-reducing activity was demonstrated for maize roots (*Marschner* and *Römheld*, 1994), this is not inducible by Fe deficiency (as occurs in dicots) and did not mobilize Fe oxide-occluded phosphate. Finally, also a possible release of organic anions did not mobilize occluded phosphate. It must be concluded that maize has no constitutive or inducible mechanism to acquire occluded phosphates.

This is in contrast to white lupin that was able to enhance P uptake when Fe oxide-occluded P was applied (Fig. 2) although P deficiency in the untreated control (3.69 mg P kg^{-1} dry mass) was not apparent in contrast to maize. The significant acquisition potential (Fig. 2) was not only reflected in enhanced growth (Fig. 1) but also in significant soil depletion of Fe oxide-occluded phosphate (Fig. 3). Several traits of white lupin may have contributed to the mobilization of Fe oxide-occluded phosphate. First, the strategy of cluster root formation allows the formation of a micro-compartment where

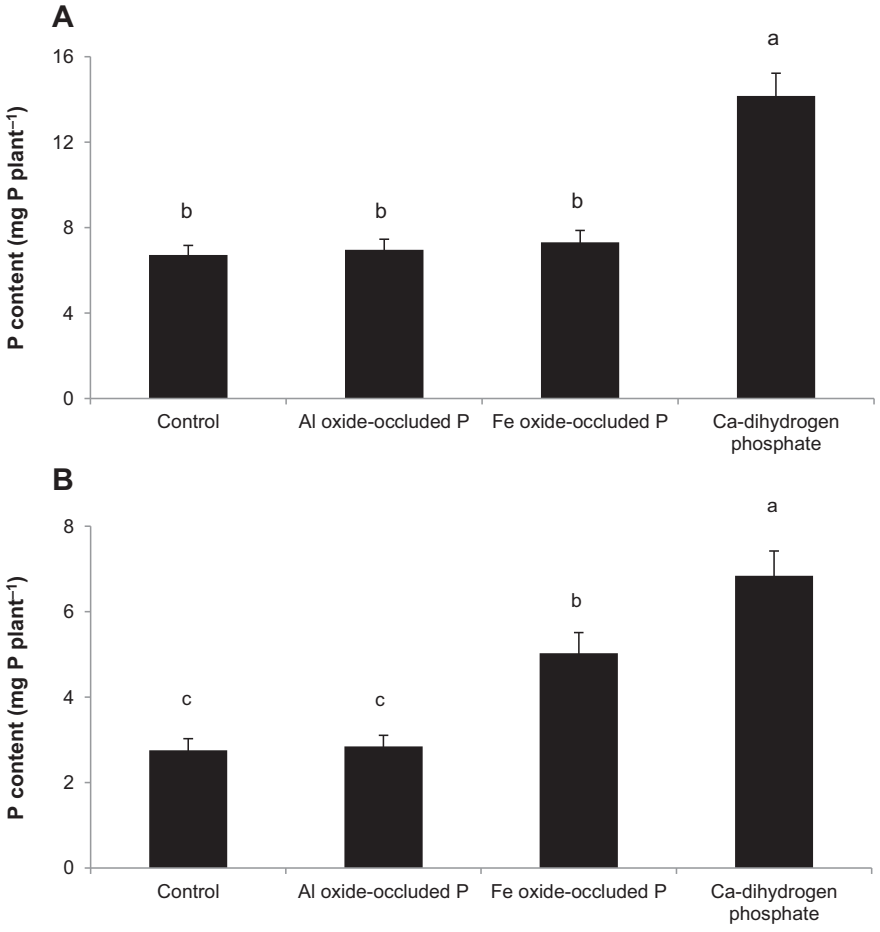

Figure 2: Effect of various phosphate sources on the P content of maize (A) and white lupin (B) after 35 d cultivation in a Luvisol subsoil (one plant kg^{-1}). In comparison to a control treatment (without P application), 10 mg P kg^{-1} soil were added as Al oxide-occluded P, Fe oxide-occluded P, or $Ca(H_2PO_4)_2$. Values are means of four replicates ± SE. Columns with different letters indicate significant differences at *P = 5%.

exudates can be concentrated (*Gardner* et al., 1982; *Neumann* and *Martinoia*, 2002; *Gerke*, 2015). Second, the difference in mobilization potential between Fe and Al oxide-occluded phosphates indicates that reduction of Fe^{III} to Fe^{II} was probably involved in the destabilization of Fe oxides surrounding the phosphates, whereas no such mobilization was possible in the case of Al oxides because the latter cannot be reduced. Cluster roots of white lupin can secrete phenols that may contribute to reduction (*Raghothama*, 1999; *Neumann* et al., 2000) and they have an inducible reductase (*Marschner* and *Römheld*, 1994). Whatever the prevalent mechanism of reduction, it is likely that white lupin is capable of mobilizing Fe oxide-occluded phosphate due to its reducing potential.

5 Conclusions

In contrast to maize, white lupin is capable of acquiring Fe oxide-occluded phosphate but not Al oxide-occluded phosphate. Further studies should unravel whether the formation of cluster roots is a precondition and/or phenol secretion or reductase activity is the major mechanism for Fe reduction. The use of plants to mobilize Fe oxide-occluded phosphate in highly weathered acid soils such as Ferralsols could be an efficient tool to mine the soil for a huge phosphate fraction that has been regarded as unavailable.

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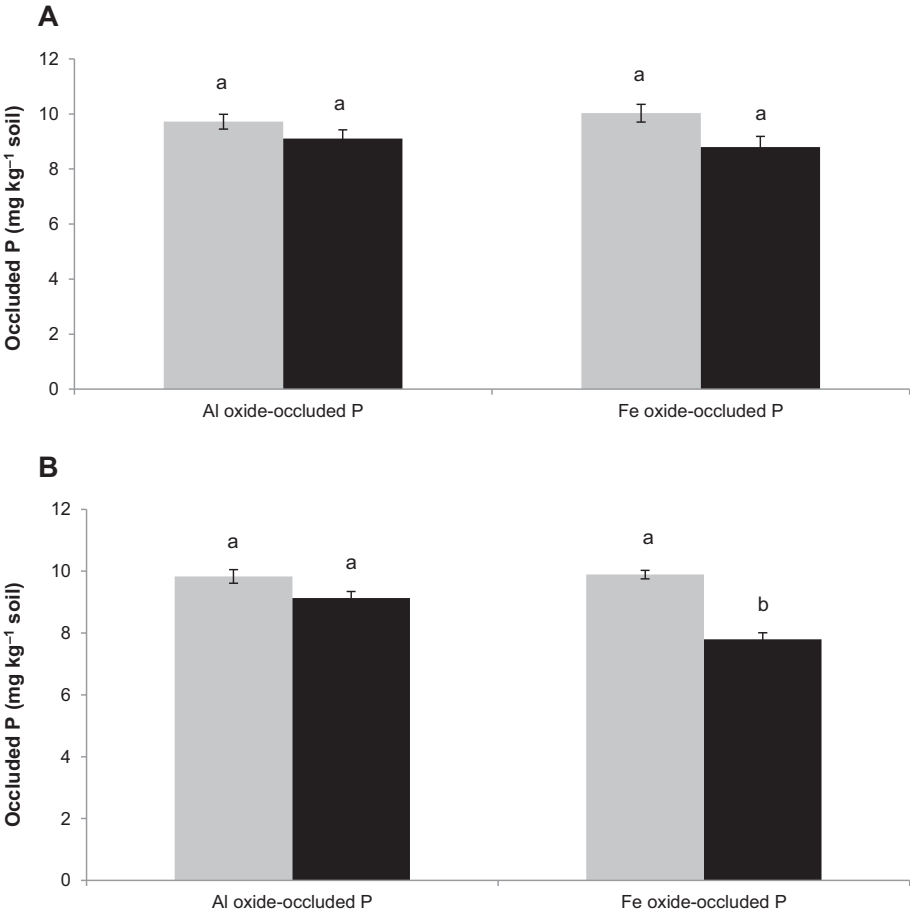

Figure 3: Effect of application of occluded P on the soil concentrations of occluded P before (gray columns) and after (black columns) cultivation (35 d) of maize (A) and white lupin (B) in a Luvisol subsoil (one plant kg^{-1}). Ten mg P kg^{-1} soil were added as Al oxide-occluded P or Fe oxide-occluded P. Values are means of four replicates ± SE. Columns with different letters indicate significant differences at *P = 5%.

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