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Elevated CO_2 temporally enhances phosphorus immobilization in the rhizosphere of wheat and chickpea

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Abstract
***Aims*:** The efficient management of phosphorus (P) in cropping systems remains a challenge due to climate change. We tested how plant species access P pools in soils of varying P status (Olsen-P 3.2-17.6 mg kg^{-1}), under elevated atmosphere CO_2 (eCO_2).
***Methods*:** Chickpea (*Cicer arietinum* L.) and wheat (*Triticum aestivum*. L.) plants were grown in rhizo-boxes containing Vertosol or Calcarosol soil, with two contrasting P fertilizer histories for each soil, and exposed to ambient (380 ppm) or eCO_2 (700 ppm) for 6 weeks.
***Results*:** The $NaHCO_3$-extractable inorganic P (Pi) in the rhizosphere was depleted by both wheat and chickpea in all soils, but was not significantly affected by CO_2 treatment. However, $NaHCO_3$-extractable organic P (Po) accumulated, especially under eCO_2 in soils with high P status. The NaOH-extractable Po under eCO_2 accumulated only in the Vertosol with high P status. Crop species did not exhibit different eCO_2-triggered capabilities to access any P pool in either soil, though wheat depleted $NaHCO_3$-Pi and NaOH-Pi in the rhizosphere more than chickpea. Elevated CO_2 increased microbial biomass C in the rhizosphere by an average of 21%. Moreover, the size in Po fractions correlated with microbial C but not with rhizosphere pH or phosphatase activity.
***Conclusion*:** Elevated CO_2 increased microbial biomass in the rhizosphere which in turn temporally immobilized P. This P immobilization was greater in soils with high than low P availability.

***Key words*:** Climate change; elevated CO_2; microbial biomass C; P fractions; phosphatase; rhizosphere acidification

Introduction

Phosphorus (P) deficiency is estimated to affect about 5.7 billion ha of arable land around the world (Hinsinger et al. 2011). In many Australian soils, P is the most limiting nutrient to agricultural production as these soils are typically ancient and highly weathered (White 1986; Lambers et al. 2006). Commercial P fertilizers are therefore regularly applied to maintain and increase plant production (McBeath et al. 2007; Simpson et al. 2011).

Long-term P fertilization has resulted in the accumulation of soil P pools. The application of P fertilizers increases the labile inorganic P (Pi) and moderately labile Pi (Beck and Sanchez 1994; Zhang et al. 2004). Furthermore, Vu et al. (2010) compared P fractions among Calcarosol, Vertosol and Chromosol soils with a long history of P fertilization, and found that the added P was transferred into all P fractions, and that the proportion of the P transferred to individual fractions depended on soil type. They reported significant increases of water-Pi fraction in the Calcarosol, the bicarbonate-Pi in the Vertosol and the NaOH-Pi in the Chromosol.

Plants normally deplete labile inorganic phosphate (Pi) in the rhizosphere since it is the only form that plants can directly utilise (Marschner 1995). However, many plant species can alter Pi availability in the rhizosphere. Different forms of Pi can be desorbed/dissolved by acidification or chemically displaced by root exudates. Plants can also mobilize organic P (Po) by producing/releasing phosphatase enzymes (Attiwill and Adams 1993). Some studies have shown that even recalcitrant P pools (typically extractable with strong mineral acids) could be decreased by growing plants (Pheav et al. 2003; Wang et al. 2007; Vu et al. 2008), indicating that either plants could directly access non-labile P pools, or non-labile pools were in chemical equilibrium with labile pools.

Plant species have various capabilities to access P in P-deficient soils. For example, chickpea exudes large amounts of low-molecular weight carboxylates, which mobilize P by competing for the same adsorption sites in the soil matrix (Gerke et al. 2000; Wouterlood et al. 2005), while wheat can utilize sparingly soluble P such as $AlPO_4$ because of its extensive root system (Pearse et al. 2006; Wang et al. 2010; 2011). Plant species respond differently to eCO_2 which could also affect their utilization of soil P. Legumes with symbiotic N_2 fixation can fix more carbon and have stronger growth response than non-legumes when exposed to eCO_2, due to the limitation in N supply to the non-legume (Overdieck 1986; Pitelka 1994; Zanetti et al. 1996; Stöcklin and Körner 1999). Moreover, rhizosphere acidification under N_2-fixing legumes may also enhance P mobilization in soil. It appears that the rhizosphere processes mediated by eCO_2 favor P acquisition in legumes more than non-legumes.

When plants are exposed to an eCO_2 environment, changes in root growth and rhizosphere processes could alter the size of soil P pools. Elevated atmospheric CO_2 increases photosynthesis in many species and this could alter carbon allocation to the roots (Campbell and Sage 2002; BassiriRad et al. 2001). Since carbohydrate supply to the roots controls their development (Roitsch 1999; Laby et al. 2000), higher sugar concentrations under eCO_2 may enhance the size of root systems (Watt and Evans 1999). Increased root growth and changes in morphology of root systems could enable plants to access new regions of the soil to promote P acquisition. On the other hand, eCO_2 may qualitatively and quantitatively change carbon-rich compounds in root exudates (Paterson et al. 1997; Hodge and Millard 1998), and these compounds could in turn stimulate microbial activity of rhizosphere (Richardson 2001; Richardson et al. 2009,2011), thereby increasing microbial demand for P and altering P availability. Changes in P availability could influence plant response to eCO_2 (Stöcklin and Körner 1999; Jin et al. 2012). However, little information is available on how eCO_2 affects the ability of plants to utilize P fractions in soils with different P status.

The objectives of this study were to (1) investigate how eCO_2 affects two contrasting plant species in the way they access P fractions in soils with different P status, and (2) explore possible mechanisms underlying these changes in soils. We hypothesised that plants under eCO_2 would access moderately or even non-labile P pools by enhancing the microbial biomass and phosphatase activity, and by altering pH in rhizosphere. Moreover, we proposed that the chickpea would utilize more non-labile P under eCO_2 than wheat, due to acidification in its rhizosphere.

Materials & Methods

Experimental design

An experiment using rhizo-boxes was conducted in a glasshouse at the Department of Primary Industries in Horsham, Victoria, Australia. The experiment consisted of a factorial design comprising two CO_2 levels, two soils with two different P status, and two plant species with four replications. The two CO_2 levels were ambient CO_2 (aCO_2; 380 ppm) and elevated CO_2 (eCO_2; 700 ppm). The plant species were chickpea (*Cicer arietinum* L. cv. Genesis 836) and wheat (*Triticum aestivum*. L. cv. Beaufort).

Soils

Vertosol and Calcarosol soils (Isbell 1996) or Vertisol and Calcic Xerosol (FAO-UNESCO 1976) differing in chemical and physical properties were used in this study (Table 1). These

are representative soil types in the dryland cropping region of southern Australian. For each soil type, paired samples were collected at a depth of 0-30 cm from plots in cropping trials with a long-term P fertiliser application history and from adjacent areas under native vegetation that had not received any P fertiliser.

The Vertosol was collected near Horsham, Victoria, Australia (36°42'S, 142°11'E). The soil with a high P status was collected from a paddock growing lentils, which had received an average of 8.8 kg P ha^{-1} per crop, applied as monoammonium phosphate, for more than 30 years. It had an Olsen-P soil test value of 17.6 mg P kg^{-1}. The native soil with a low P status was collected from an undisturbed fence line, and had an Olsen-P of 3.2 mg P kg^{-1} soil.

The Calcarosol was collected from the Mallee Research Station of the Department of Primary Industries Victoria, Walpeup, Australia (35°07′S, 141°59′E). The soil with a high P status was from a plot of a wheat-fallow-fallow rotation since 1940. The plot received 12 kg P ha^{-1} as a single superphosphate with every wheat crop in this long-term experiment (Vu et al. 2008). Olsen-P in the soil was 16.0 mg P kg^{-1} soil. A control plot within the same experiment was used as the soil with a low P status and had Olsen-P of 4.4 mg P kg^{-1}.

Rhizo-box

A rhizo-box device used in the experiment was modified from a design used by Armstrong and Helyar (1992). A thin soil layer (10 mm) was established by packing 100 g of sieved (≤4 mm) air-dry soil into a black Perspex container (100 mm wide × 150 mm high × 10 mm thick; rhizo-box) that was open at the base. The soil within the rhizo-box was mixed with P-free basal nutrients (mg kg^{-1}): urea, 60; K_2SO_4, 147; $MgSO_4.7H_2O$, 122; $CaCl_2$, 186; $CuSO_4.5H_2O$, 6; $ZnSO_4.7H_2O$, 8; $MnSO_4.5H_2O$, 6; $FeCl_3$, 0.6; $CoCl_2$, 0.4; $NaMoO_4.2H_2O$, 0.4; and NaB_4O_7, 1.6. Water and basal nutrients were supplied to the rhizo-box using a sand bath. Briefly, each rhizo-box was placed upright on top of a pot containing 2 kg washed sand that received 30 mg kg^{-1} of urea in addition to the basal nutrients above. The inside wall of each rhizo-box was lined with muslin which extended into the sand bath to facilitate water and nutrient movement between the two compartments.

Plant growth and sampling procedures

Seeds of chickpea and wheat were germinated for 1 d between moistened filter paper. Four geminated seeds of chickpea or 5 of wheat were sown in each rhizo-box. Plants were grown in naturally light glasshouse compartments with either aCO_2 or eCO_2 and air temperature set at a constant 23°C. For each treatment, two rhizo-boxes without plants were also included as bulk soil controls.

After 6 weeks of growth rhizo-boxes were destructively sampled for plant and soil measurements. Plant shoots were cut at ground level and washed with 0.1 M HCl and then rinsed twice in deionized water to remove dust particles (Tang et al. 1990). The rhizo-box was opened and roots were carefully removed. Most of the soil in the rhizo-box was adhered to roots. The rhizosphere soil was recovered by gently shaking the roots. The soil collected was kept cold using ice during sampling and stored at 4°C overnight. Roots were then washed with deionized water to remove any remaining soil. The root morphology was determined using the WinRhizo Pro version 2003b programme (Régent Instruments Inc., Québec, CA). All plant material was dried at 70°C for 72 h and the dry weights were recorded. Subsamples of the root and shoot material were finely ground (≤0.2 mm) and digested with concentrated nitric and perchloric acids (4:1) (Yuen and Pollard 1954). The P

concentration in the digests were determined colorimetrically using malachite green (Motomizu et al. 1983).

Microbial biomass C (MBC) and phosphatase activity in rhizosphere soil were assessed in the following day. The MBC was determined by chloroform fumigation-extraction according to Vance et al. (1987). Total organic C (TOC) in the extracts was determined following dichromate oxidation (Heanes 1984; Conyers et al. 2011). Total MBC was calculated as the difference in TOC concentration between fumigated and non-fumigated soils and using a k_{EC} of 0.37 (Joergensen, 1996). Activities of acid and alkaline phosphatases were determined by measuring the release of para-nitrophenol from para-nitrophenyl phosphate after adding the soil to a modified universal buffer (MUB) (Skujins et al. 1962) at pH 6.5 for acid phosphatase and pH 11 for the alkaline phosphatase (Tabatabai and Bremner 1969).

The remainder of the soils were air-dried and milled to <0.5 mm for pH analysis and P fractionation. Soil pH was determined with a Thermo Orion 720 pH meter after shaking soil (1:5 w/v) in 0.01 M $CaCl_2$ solution for 17 h followed by centrifuging at 500 *g* for 10 min. Phosphorus fractionation was performed using a modified Hedley P fractionation scheme (Guppy et al. 2000). Inorganic P (Pi) within the fractions was determined using malachite green (Motomizu et al. 1983). Total dissolved P in bicarbonate ($NaHCO_3$) and hydroxide (NaOH) fractions were determined following digestion using acid ammonium persulphate and by autoclaving (103 kPa and 121°C) for 1 h (Butterly et al. 2009). The Po in these two fractions was calculated by subtracting the Pi from the total P.

Statistical analysis

Data were analysed with Genstat 13 (VSN International, Hemel Hemspstead, UK). Analysis of variance (ANOVA) was used to determine the effects of P status, CO_2 and species, and their interactions on plant biomass, root morphology, P and N concentrations, pH, phosphatase activity and soil P fractions. A Tukey Honest Significant Differences test was used to assess the differences between treatment means (Steel and Torrie 1980).

Results

Shoot and root growth

Elevated CO_2 and a previous P fertilizer application (high P status) resulted in significantly ($P < 0.01$) greater shoot biomass for chickpea and wheat in the Vertosol and wheat in the Calcarosol (Figure 1A and Table 2) than their respective control treatments. A significant $CO_2 \times P$ interaction was found in the Vertosol, with chickpea shoot biomass showing a 25% increase by eCO_2 in the soil with high P status compared to a 6% increase in the low-P soil. Similarly, wheat showed a 41% increase in shoot biomass under eCO_2 in the high-P soil compared to an 18% increase in the Vertosol with low P (Figure 1A). In the Vertosol soil, the positive responses of wheat growth to eCO_2 and to P application were greater than those of chickpea, leading to significant $CO_2 \times$ species and P × species interactions. In the Calcarosol soil, however $CO_2 \times P$, $CO_2 \times$ species and P × species interactions were not significant (Table 2).

Root biomass of both wheat and chickpea grown in the Vertosol had a positive response to eCO_2 and to the high soil P status (Figure 1B). A 51% increase in root biomass under eCO_2 in the soil with high P status compared with only 2% increase when no P was applied, leading to a significant $CO_2 \times P$ interaction ($P < 0.001$) (Table 2). Such an interaction did not occur in the Calcarosol although root biomass was higher in the soil with high P status.

The CO_2 treatments did not affect the root-to-shoot ratio irrespective of species and soil type (Table 2). In the Calcarosol soil, high P status resulted in a 30% decrease in the root-to-shoot ratio of wheat but did not significantly change that of chickpea (Figure 1C). In the Vertosol, P status did not affect the root-to-shoot ratios of either species (Figure 1C; Table 2).
Elevated CO_2 and high P status greatly increased root length (data not shown). The increase in root length under eCO_2 was greater in the Vertosol with high than with low P status, resulting in the CO_2 × P interaction ($P < 0.05$) (Table 2). In the Calcarosol, eCO_2 did not significantly change root length of either species (Table 2) whereas previous P fertilizer application (high P status) increased root length of chickpea and wheat by 34% and 32%, respectively (data not shown).

Phosphorus concentrations and P uptake

Shoot P concentrations of both chickpea and wheat were significantly ($P < 0.001$) higher in both soils with high than with low P status, but were not significantly affected by eCO_2 (Figure 2A, Table 2). The history of P fertilizers (high P status) increased shoot P concentration more in wheat than in chickpea, contributing to significant P × species interactions (Figure 2A, Table 2). A significant CO_2 × P interaction on shoot P concentration was observed in the Calcarosol; the P concentration being 18% lower under eCO_2 than under aCO_2 in soils with high P status, but not affected by CO_2 treatment in the soil with low P status.

Elevated CO_2 did not affect root P concentrations of either species while the high P status generally increased root P concentrations of both species (Figure 2B). There were no significant CO_2 × P, CO_2 × species, P × species and CO_2 × P × species interactions (Table 2).

A significant CO_2 × P interaction occurred on total P uptake when plants were grown in the Vertosol, but not in the Calcarosol (Figure 2C, Table 2). In the Vertosol with high P status, eCO_2 increased total P uptake of chickpea and wheat by 32% and 57%, respectively, but there was no change in the soil with low P status. Chickpea had greater P uptake than wheat (Figure 2C) for similar treatments. Furthermore, previous P application (high P) in the Vertosol increased total P uptake by 2.1-fold for wheat compared to 1.5-fold for chickpea (Figure 2C) and this was the basis for the P × species interaction (Table 2).

Elevated CO_2 did not affect P uptake per unit root length, while the previous P application (high P status) increased it from 0.08 to 0.13 mg P m^{-1}. The P uptake per unit root length of chickpea was 2.9-fold higher than that of wheat (data not shown).

P fractionation in rhizosphere soil

Compared with the bulk soil of the non-planted control, plants depleted $NaHCO_3$-Pi in the rhizosphere (Figure 3A) and the depletion also occurred on NaOH-Pi in the rhizosphere of wheat in the Vertosol (Figure 3C). Organic P extracted by $NaHCO_3$ ($NaHCO_3$-Po) accumulated in the rhizosphere (Figure 3B), but no change was observed for NaOH-Po in the rhizosphere compared to the bulk soil when plants grown under aCO_2 (Figure 3D).

Soil with high P status had significantly ($P < 0.001$) greater concentrations of Pi and Po in both $NaHCO_3$ and NaOH fractions (Figure 3). However, eCO_2 only increased the $NaHCO_3$-Po and NaOH-Po fractions in the Vertosol soil and the $NaHCO_3$-Po in the Calcarosol soil (Figure 3B, D), with the increases only occurring in the soil with high P status (Table 3). The concentration of $NaHCO_3$-Pi was higher in the rhizosphere of chickpea than wheat irrespective of CO_2 treatment (Figure 3A) but this only occurred in soils with high P status. This was the basis for the P × species interaction in both soils (Table 3).

Atmospheric CO_2 treatments did not alter HCl-P or residual-P fractions in the rhizosphere of either crop species. Compared with low P status, high P status resulted in an increase in HCl-P from 12.4 to 30.5 mg P kg^{-1}, and residual-P from 85.2 to 95.6 mg P kg^{-1} (data not shown).

Microbial biomass C, phosphatase and pH in the rhizosphere
In general, MBC was 16-50% greater in the rhizosphere of chickpea than the bulk soil, while the increased MBC in wheat rhizosphere occurred only under eCO_2 (Figure 4). Elevated CO_2 and high P status increased MBC in the rhizosphere by 21% and 55%, respectively (Figure 4A, Table 3). Similarly, there was higher MBC observed in the rhizosphere of chickpea than of wheat, irrespective of soil type (Figure 4A, Table 3). Moreover, MBC was positively correlated with $NaHCO_3$-Po (R^2 =0.50−0.80) (Figure 5A) and NaOH-Po (R^2 = 0.56−0.60) irrespective of CO_2 and P treatments (Figure 5B).

Phosphatase activity was 1.3 to 4.2 times greater in the rhizosphere soil than in the bulk soil (Figure 4B, C). Acid phosphatase activity was not significantly influenced by either CO_2 treatment or soil P status (Figure 4B, C; Table 3). Interestingly, alkaline phosphatase activity decreased in the rhizosphere of chickpea in the Vertosol with high P status, but such observation did not occur in wheat (Figure 4C); this resulted in the significant P × species interaction (Table 3).

The rhizosphere pH was 0.2−1.5 pH units lower than bulk soil pH (Figure 4). Rhizosphere pH was, on average, 0.3 units higher under eCO_2 than aCO_2 for wheat grown in the Vertosol and 0.5 units higher for chickpea and wheat in the Calcarosol (Figure 4D). There was no significant interaction between CO_2 and P or between CO_2, P and species (Table 3).

Discussion

This study revealed that eCO_2 significantly changed organic P fractions in the rhizosphere of both a cereal (wheat) and pulse (chickpea), in a Vertosol and Calcarosol with high P status. In particular, there was an increase in organic P in the $NaHCO_3$ and NaOH fractions of the Vertosol and in the $NaHCO_3$-Po fraction of the Calcarosol (Figure 3). These results are consistent with our previous findings that eCO_2 significantly increased the NaOH-Po in the rhizosphere of chickpea and field pea when P fertilizer was applied to a P-deficient Vertosol (Jin et al. 2012).

The effect of eCO_2 on soil P availability to plants is inconsistent in the limited number of previous studies. In the FACE (Free Air CO_2 Enrichment) experiment with sweetgum (*Liquidambar styraciflua* L.), there were no significant effects of eCO_2 on ortho-P availability assessed using a resin extraction technique (Johnson et al. 2004). Similarly, Khan et al. (2008) found that the Mo-reactive P extracted in H_2O did not change after poplars (*Populus alba* L. or *Populus nigra* L.) were grown in the EuroFACE for 5 years. Other studies have shown a negative effect of eCO_2 on soil P availability. Johnson et al. (1997) showed that the HCl (0.5 M)- plus NH_4F (1 M)-extractable P was decreased after growing *Ponderosa* pine in open-top chambers enriched with 700 ppm CO_2 for 3 years. In a subsequent study, Johnson et al. (2003) reported that in a fire-adapted scrub oak ecosystem eCO_2 decreased P availability after 5 years. These contradictory results between the studies may be attributed to the different methods used for P extraction. Differences in CO_2 concentration, plant types, and experiment duration could also be responsible for disparate results. All these factors can significantly affect the quantity and quality of root exudates from plant roots and thus affect P availability in soils (Schortemeyer et al. 2000; Lambers et al. 2006). Moreover, these studies

only measured the P in the bulk soil and so any changes in the P availability in rhizosphere soil would not be detected against a large background P level in the bulk soil.

In this study, the increase in Po fractions in the rhizosphere of crops under eCO_2 indicates that P was temporally immobilized. This could be attributed to an increased microbial activity/biomass, resulting in the transformation of P between pools. Elevated CO_2 has been shown to increase root exudates and organic debris sloughing off roots (Schortemeyer et al. 2000), which enhanced microbial turnover in the rhizosphere (Rice et al. 1994). In our study, MBC in the rhizosphere in the soils with high P status was markedly higher under eCO_2 than under aCO_2 (Figure 4A, Table 3), suggesting that microbial activity and growth in the rhizosphere was likely stimulated by eCO_2. Furthermore, a significant correlation between MBC and the Po fractions (Figure 5) supports this view. A Switzerland FACE study by Montealegre et al. (2002) showed that elevated CO_2 resulted in an 85% increase in total bacteria and a 170% increase in respiring bacteria in the rhizosphere of white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.). Furthermore, eCO_2 (540 ppm) increased microbial growth rate by 58% in the rhizosphere of *Populus deltoides* grown in a Cambisol (Blagodatskaya et al. 2010). Further experimental evidence, like specific microbial species responsible for the transformation between P pools in the rhizosphere under eCO_2 is still needed.

It should be noted that the Po accumulation in the rhizosphere under eCO_2 was dependent on soil P status with Po accumulation being greater in soils with high P status. There are two possible reasons for this finding. First, root exudates were likely to increase under eCO_2 and these could be P-enriched. These P-enriched root exudates could contribute to the Po pool in the rhizosphere. Second, increased microbial immobilisation of labile P due to a higher P availability in the soils with high P status (Table 1) and increased supply of readily available C from root exudates under eCO_2 could result in an increase in the Po pool. Microbial biomass P constitutes a significant portion, amounting to around 2-10% of the organic P (Binkley et al. 2000; Achat et al. 2010; Richardson and Simpson 2011). It is likely that the larger microbial biomass in soils with high P status (Figure 4A, Table 3) would immobilise more labile P under eCO_2. However, the origins of the increased Po in the rhizosphere soil under eCO_2 need to be better understood in terms of transformations in the root-soil-microbe continuum. It is difficult to distinguish the roles of increased root growth and enhanced microbial biomass production in increasing Po content in the rhizosphere in this study.

Interestingly, the NaOH-Po fraction only accumulated in Vertosol and not in the Calcarosol (Figure 3D, Table 3). This indicates that soil type can affect the response of the Po pools to eCO_2. This difference in NaOH-Po accumulation between the soils could reflect the different soil physicochemical properties influencing P transformations. The Vertosol had higher clay content than the Calcarosol (Table 1), and this could have resulted in the accumulation of P-enriched organic compounds. Clay can bind and protect organic compounds including amino acids and peptides, and prevent them undergoing microbial degradation. The organic compounds can be entrapped in the small ($1 < \mu m$) pores formed between clay particles such that the compounds are physically inaccessible to decomposing microbes (Swinnen et al. 1994; Hütsch et al. 2002).

Phosphatases play an essential role in the transformation from Po to Pi (George et al. 2002) and phosphatase activity could increase under eCO_2 due to the greater P demand via enhanced plant growth (Prior et al. 1994; Barrett et al. 1998). In this study, however, eCO_2 did not affect the activity of acid or alkaline phosphatase in either soil (Figure 4B, C),

indicating that phosphatase production was not stimulated to mobilise the Po fractions formed under eCO_2 during the experimental period. Future research is needed to verify this. Nevertheless, a study at a tundra site showed that eCO_2 increased the amount of available P by 3 fold due to increasing phosphatase activity on the root surface of *Eriophorum vaginatum* (Moorhead and Linkins 1997). The long-term adaptation of the local plant community to low P availability in soils with high organic matter (>117 g C kg^{-1} soil) in the arctic tundra ecosystem can explain this result (Moorhead and Linkins 1997; Dai et al. 2002). This was not the case for the crop plants in this study where soils with lower organic matter content were used (Table 1). However, it is expected that the increased Po fractions under eCO_2 have potential to be mineralized into labile Pi.

There were minimal differences between the two crop species in the size of the P fractions in their rhizosphere in this study. Wheat significantly depleted NaOH-Pi in the rhizosphere in the Vertosol with high P status, whereas chickpea did not (Figure 3C, Table 3). It is expected that rhizosphere acidification would stimulate P mobilization from non-labile P pools (Lambers et al. 2006), but the pH in the wheat rhizosphere did not change in comparison with the bulk soil of Vertosol. Although the chickpea grown in the Calcarosol acidified its rhizosphere by 1 pH unit, compared to the bulk soil pH (Figure 4C), the soil pH in the rhizosphere was not low enough to mobilize P (Grinsted et al. 1982).

Root morphology is likely to be one of the main contributors for the greater depletion of NaOH-Pi in the rhizosphere of wheat. Wheat had a comparatively smaller root diameter and greater specific root length (root length per unit root biomass) than chickpea (data not shown), which permits a larger volume of soil to be explored per unit of root mass (Veneklaas et al. 2003). Thus, the finer root system of wheat compared with that of chickpea is likely to increase the chance of intercepting Al-P and Fe-P soil minerals in the soil and mobilizing these moderately labile P forms, more efficiently, especially in the Vertosol which has a higher P buffering capacity (Table 1) and Al and Fe contents (Vu et al. 2009; Wang et al. 2011).

Conclusion

Predicting the P fertilizer requirements for maximum production of crops with increasing CO_2 concentrations requires an understanding of how crop species access P pools in soils. Although elevated CO_2 did not alter the labile Pi pool ($NaHCO_3$-Pi) in the rhizosphere of either wheat or chickpea at the time of harvest, it increased plant P uptake. Interestingly, the Po fractions accumulated in the rhizosphere when plants were grown under eCO_2, particularly in soils with high P status. There was no obvious difference between wheat and chickpea in depleting different soil P pools in an eCO_2 environment although chickpea tended to decrease rhizosphere pH. An increase in MBC but not phosphatase activity under eCO_2 was the likely cause of the increases in soil Po pools. Further studies are needed to underpin the specific function of microbes on the P transformations between P pools in the rhizosphere soil when plants are grown under eCO_2.

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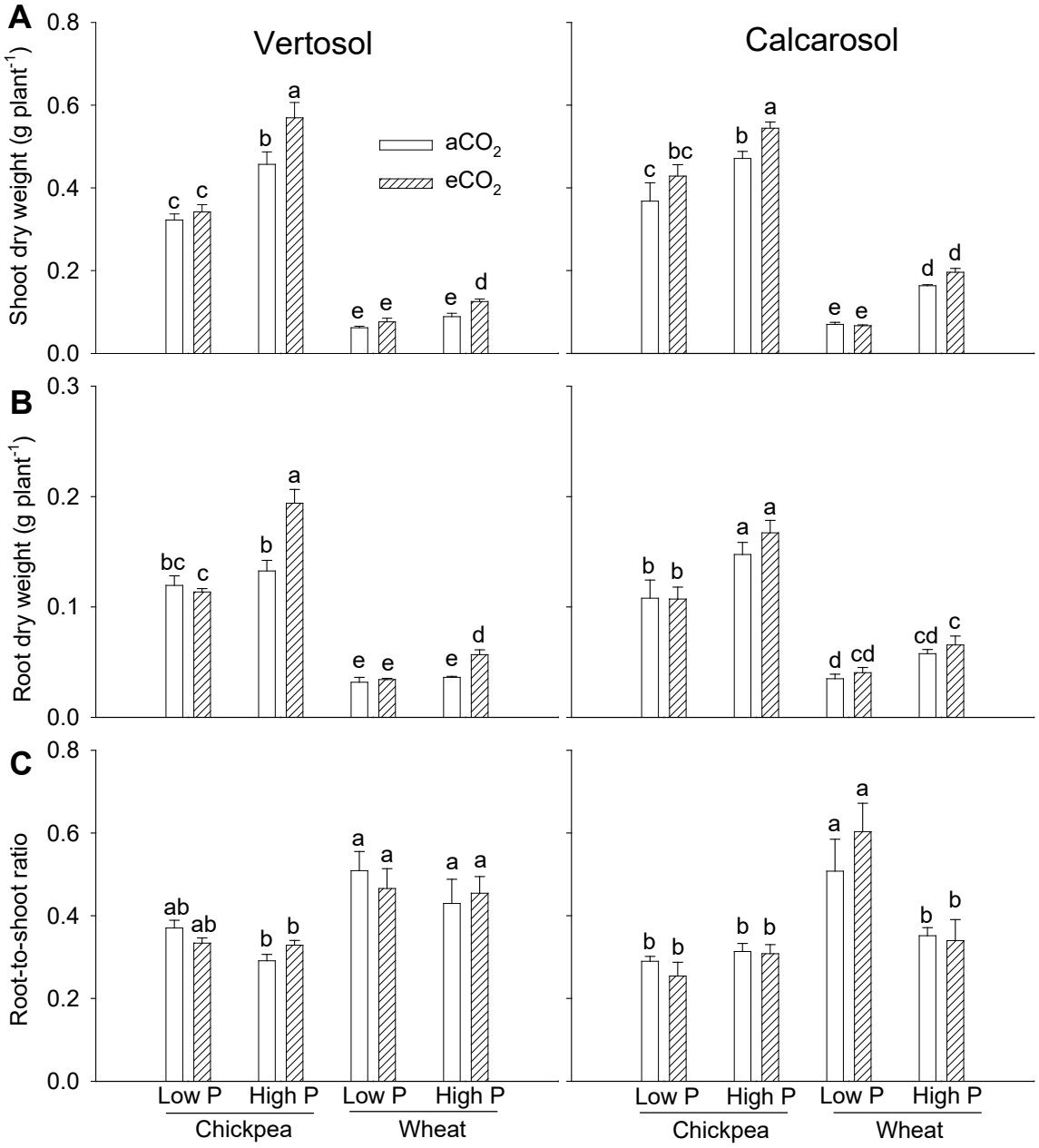

Figure 1. The effect of eCO_2 on shoot biomass (A), root biomass (B), and the root-to-shoot ratios (C) of chickpea and wheat grown in Vertosol (left) or Calcarosol (right) with low and high P status. All plants were grown in rhizo-boxes supplied with nutrients (except for P) for 6 weeks under 380 or 700 ppm CO_2. Values were means ± SE (n=4). Values with a same letter are not significantly different (P = 0.05).

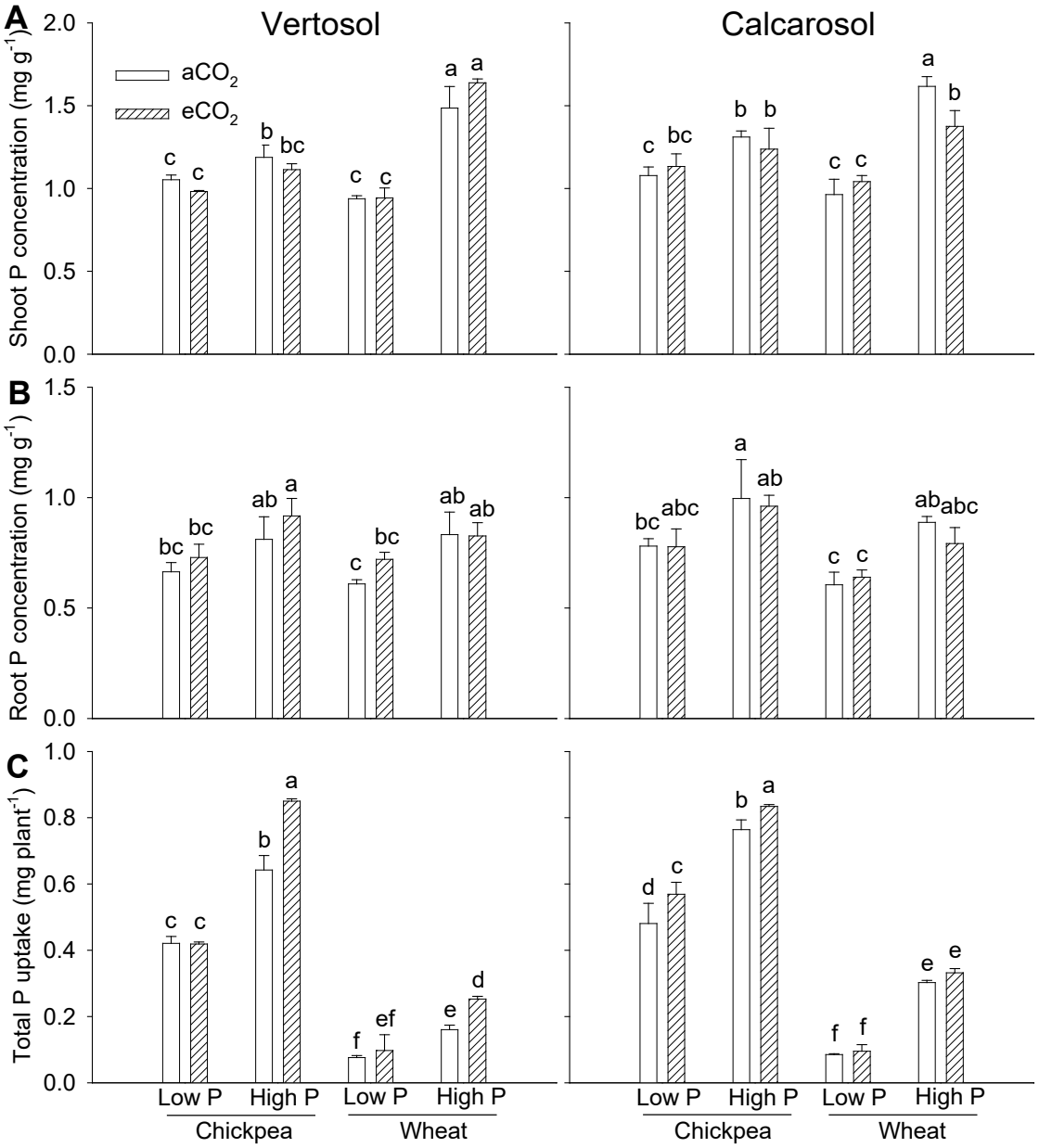

Figure 2. The effect of eCO_2 on P concentrations in shoot (A) and root (B), and total P uptake (C) of chickpea and wheat grown in Vertosol (left) or Calcarosol (right) with low or high P status . All plants were grown in rhizo-boxes supplied with nutrients (except for P) for 6 weeks under 380 or 700 ppm CO_2. Values were means ± SE (n=4). Values with a same letter are not significantly different (P = 0.05).

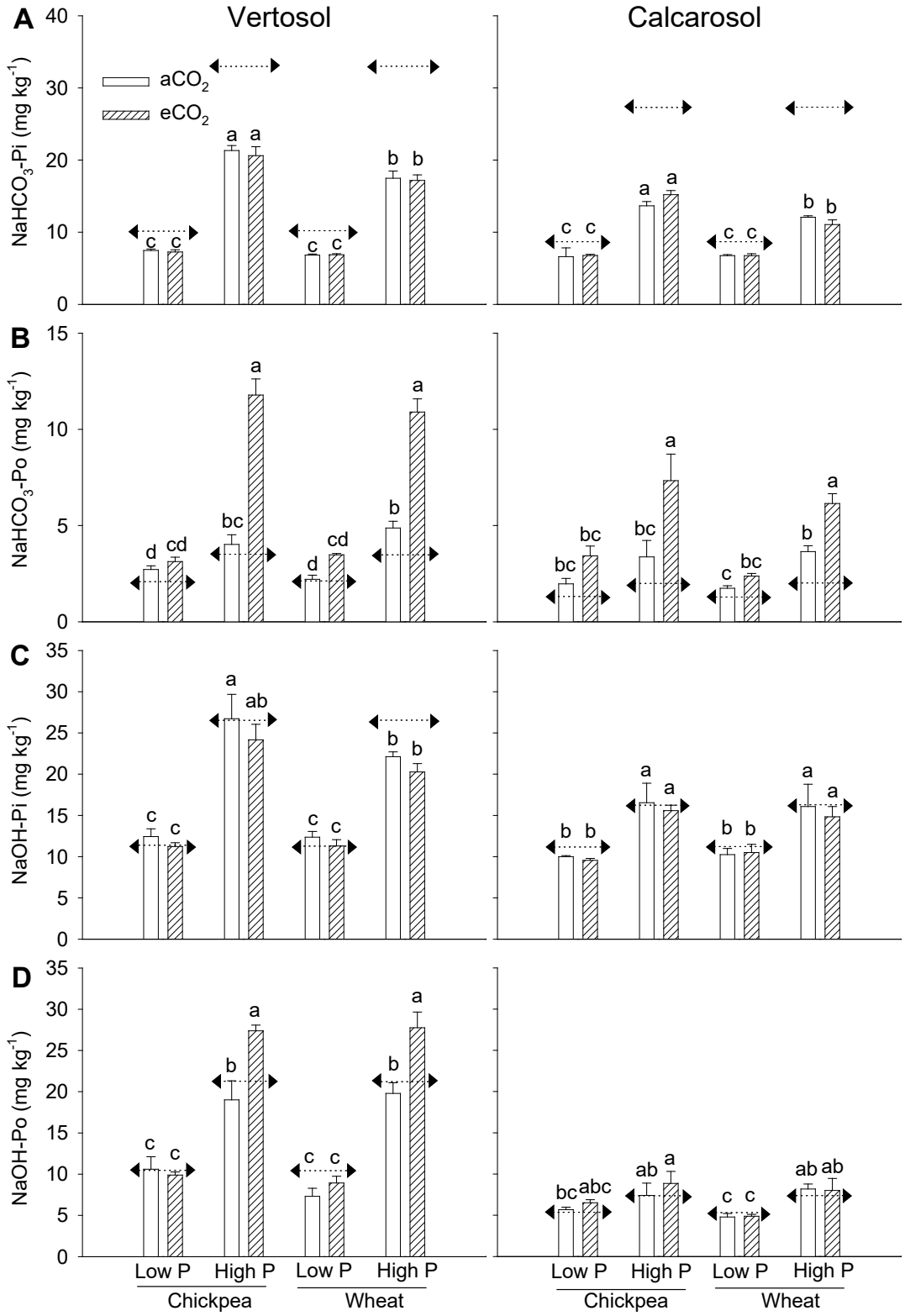

Figure 3. The effect of eCO_2 on concentrations of $NaHCO_3$-Pi (A), $NaHCO_3$-Po (B), NaOH-Pi (C) and NaOH-Po (D) in rhizosphere soil of chickpea and wheat grown in Vertosol (left) or Calcarosol (right) with low or high P status. All plants were grown in rhizo-boxes supplied with nutrients (except for P) for 6 weeks under ambient (380 ppm) or elevated CO_2 (700 ppm). The arrow-ended short lines represent corresponding value in each soil treatment without growing plants. Values were means ± SE (n=4). Values with a same letter are not significantly different between treatments within soil (P = 0.05).

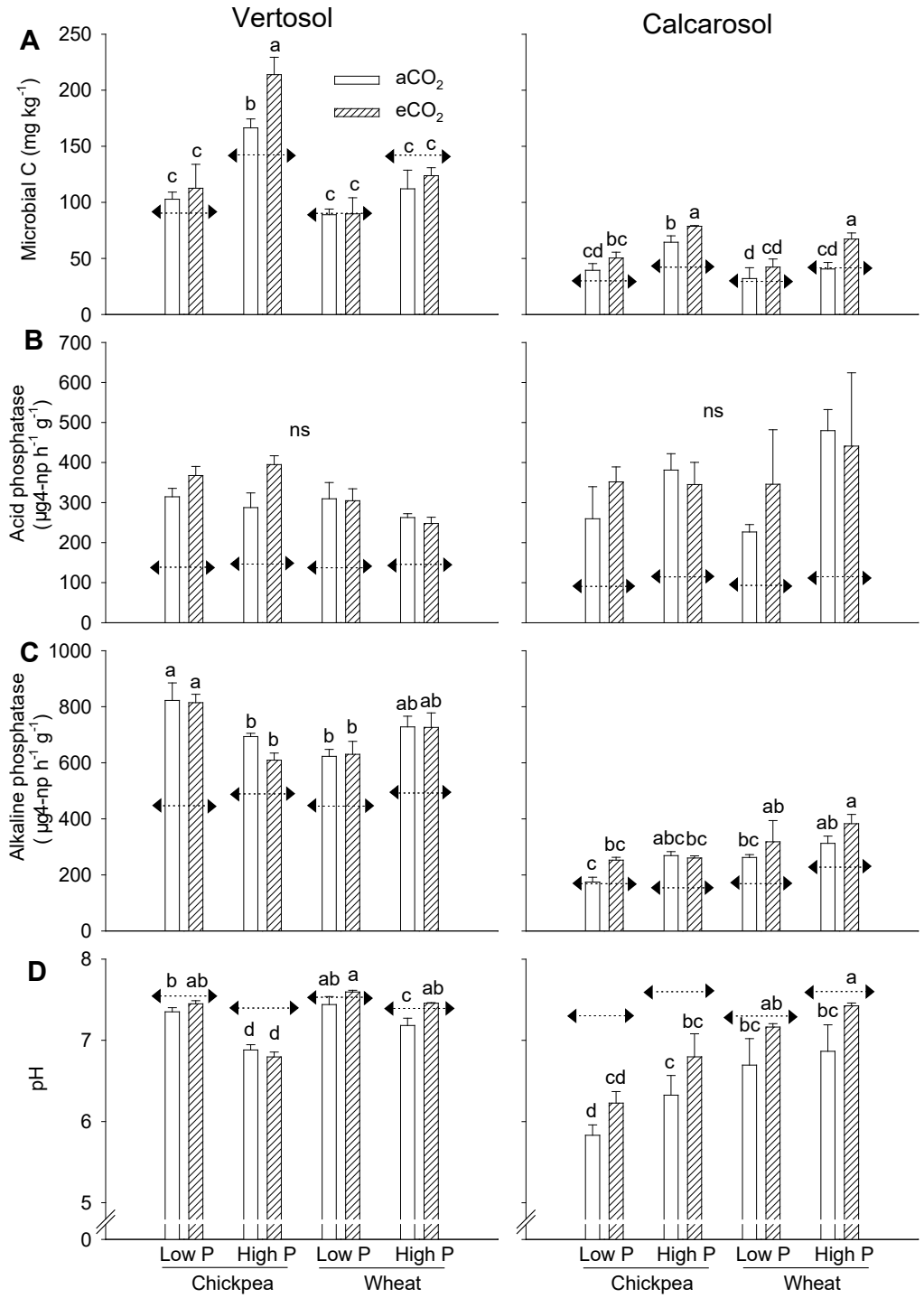

Figure 4. The effect of eCO_2 on microbial C (A), phosphatase activity at pH 6.5 (B) and pH 11 (C), and pH (D) in the rhizosphere soil of chickpea and wheat grown in Vertosol (left) or Calcarosol (right) with low or high P status. All plants were grown in rhizo-boxes supplied with nutrients (except for P) for 6 weeks under ambient (380 ppm) or elevated CO_2 (700 ppm). The arrow-ended short lines represent the corresponding value in each soil treatment without growing plants. Values were means ± SE (n = 4). Values with a same letter are not significantly different between treatments within soil (P = 0.05). ns, No significant difference.

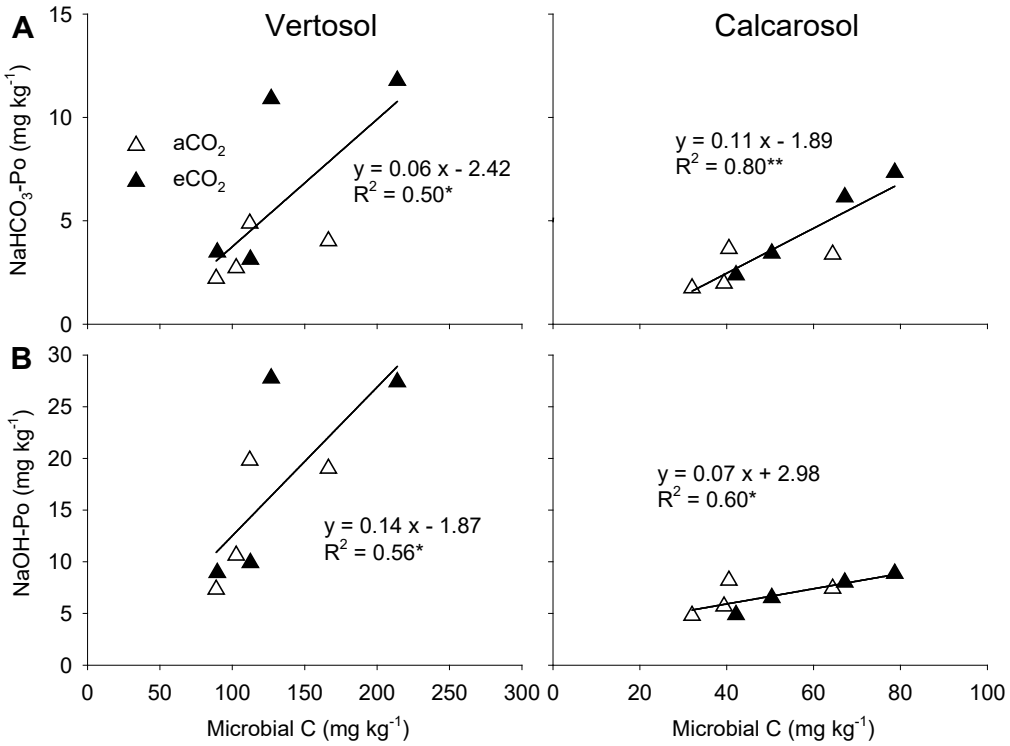

Figure 5. Relationships between microbial biomass C and $NaHCO_3$-Po (A) and NaOH-Po (B) in the rhizosphere of plants grown in Vertosol (left) or Calcarosol (right) under ambient (380 ppm) or elevated CO_2 (700 ppm) for 6 weeks. * and ** indicate significance at $P < 0.05$ and $P < 0.01$, respectively.

Table 1. General properties of the two soil types, with low or high P status, that were used in the study

Soils	pH	Total orgaic C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	Total P ($mg\ kg^{-1}$)	Olsen-P ($mg\ kg^{-1}$)	PBC[#]	Clay content (%)
Vertosol							
Low P	7.5	11.4	0.9	141	3.2	80	40
High P	7.3	13.3	1.1	254	17.6	80	37
Calcarosol							
Low P	7.5	19.9	0.3	111	4.4	76	6
High P	7.3	16.3	0.4	158	16.0	46	6

[#] represents phosphorus buffering capacity (PBC, $mg\ kg^{-1}/log_{10}\mu g\ L^{-1}$)(Rayment and Higginson, 1992)

Table 2. Significance levels for main effects and interaction terms from the analysis of variance, for CO_2, P status (P) and species on dry weights (DW) of shoots and roots, root-to-shoot ratio (R/S), root length, P concentrations (conc.) in shoots and roots and total P content per plant.

Soils	Factors	Shoot DW	Root DW	R/S	RootL	Shoot P conc.	Root P conc.	Total P
Vertosol	CO_2	***	***	-	*	-	-	***
	P	***	***	-	**	***	***	***
	Species	***	***	***	***	**	-	***
	CO_2×P	**	***	-	*	-	-	***
	CO_2×Species	*	-	-	-	-	-	-
	P×Species	***	***	-	-	***	-	***
	CO_2×P×Species	-	*	-	-	-	-	*
Calcarosol	CO_2	**	-	-	-	-	-	*
	P	***	***	*	***	***	**	***
	Species	***	***	***	**	-	*	***
	CO_2×P	-	-	-	-	*	-	-
	CO_2×Species	-	-	-	-	-	-	-
	P×Species	-	*	***	-	**	-	-
	CO_2×P×Species	-	-	-	-	-	-	-

*, **, *** and - indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ and no significance, respectively.

Table 3. Significance levels of main effects and interaction terms from the analysis of variance, for CO_2, P status (P) and species on $NaHCO_3$-Pi, $NaHCO_3$-Po, NaOH-Pi, NaOH-Po, Microbial C, rhizosphere pH, and acid (pH 6.5) and alkaline (pH 11) phosphatase activity.

Soils	Factors	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	Microbial-C	pH	Acid phosphatase	Alkaline phosphatase
Vertosol	CO_2	-	***	-	***	*	*	-	-
	P	***	***	***	***	***	***	-	-
	Species	***	-	*	-	***	***	-	-
	CO_2×P	-	***	-	***	-	-	-	-
	CO_2×Species	-	-	-	-	-	*	-	-
	P×Species	*	-	*	-	**	***	-	***
	CO_2×P×Species	-	-	-	-	-	-	-	-
Calcarosol	CO_2	-	***	-	-	***	*	-	-
	P	***	***	***	***	***	*	-	-
	Species	**	-	-	-	**	***	-	*
	CO_2×P	-	*	-	-	-	-	-	-
	CO_2×Species	-	-	-	-	-	-	-	-
	P×Species	**	-	-	-	-	-	-	-
	CO_2×P×Species	-	-	-	-	-	-	-	-

*, **, *** and - indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ and no significance, respectively.