The impact of elevated carbon dioxide on the phosphorus nutrition of plants: a review

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The concentration of CO2 in the atmosphere continues to rise. It has increased from 270 μL·L−1 prior to the Industrial Revolution to 384 μL·L−1 in 2009, and 394 μL·L−1 in 2013 (Leakey et al., 2009; Goufo et al., 2014). The rate of change of CO2 concentration has accelerated with models predicting that this century (Long and Ort, 2010; Feng et al., 2014). Thus, elevated CO2 is likely to stimulate the growth of many plant species (Poorter, 1998; Sakurai et al., 2014). However, an increase in the growth of plants will need an increased supply of essential plant nutrients. In fact, limitations in supply of nutrients such as nitrogen (N) may offset the positive effects of elevated CO2 on photosynthesis, thereby constraining species growth (Drake et al., 1997; Ainsworth et al., 2003). Decreases in N concentration in the leaf and entire plant have been associated with photosynthetic acclimation (Stitt and Krapp, 1999; Nowak et al., 2004; Ainsworth and Long, 2005). The need for extra N supply under elevated CO2 is indicated by the work of Reich et al. (2006) who found that there was a 20–25 % increase in plant biomass by elevated CO2 with enriched N, in comparison with only 8–12 % with an insufficient N supply. The impact of elevated CO2 on the N cycle in ecosystems, and on soil N mineralization and immobilization, and organic matter decomposition and turnover have been well studied (Hungate et al., 2003; Luo et al., 2004; Schneider et al., 2004; Wang et al., 2013; Xu et al., 2013). In comparison, the impact of elevated CO2 on interactions between soil P supply and plant growth need further interpretation.

Phosphorus is a unique nutrient among the essential plant nutrients with respect to increasing atmospheric CO2 concentrations, and is the focus of this review. It plays an essential role in plant metabolism as it is involved in conserving and transferring energy in cell metabolism (Raghothama, 1999; Abel et al., 2002; Lambers et al., 2006), and is an indispensable structural component of nucleic acids, coenzymes, nucleotides, phosphoproteins, phospholipids and sugar phosphates (Schachtman et al., 1998; Veneklaas et al., 2012). The growth increases from elevated CO2 are likely to require more P, which is taken up from the available P pool in soil (Edwards et al., 2005; Gentile et al., 2012; Jin et al., 2012). Several studies have reported that both the magnitude and the direction of the growth response of plants to elevated CO2 depend on P availability (BassiriRad et al., 2001; Jin et al., 2013). However, only a small proportion...
of total soil P (generally <1 %) is in the form of labile phosphorus, which are available to plants (Richardson et al., 2009). This means that the plant-available P concentrations in soils are small, despite the total P in soils being in the range 200–3000 mg P kg$^{-1}$. This presents challenges to plants in acquiring sufficient P from the soil to meet their needs.

It is not surprising then that some plants have developed specific P acquisition strategies to adapt to the small concentrations of available P forms in the soil. The first is the ability of the roots to proliferate, extend and explore the soil. This would include growing root hairs, proteoid roots (some species) and basal roots (Keerthisinghe et al., 1998; Hodge, 2004; Ramaekers et al., 2010; Haling et al., 2013). The second is to develop mycorrhizal associations, where arbuscular mycorrhizal fungi form symbioses with plant roots, with mycorrhizal hyphae increasing the P-absorbing surfaces to increase the spatial availability of P (Facelli et al., 2010; Shen et al., 2011; Brown et al., 2013). The third is to be able to modify the rhizosphere environment to increase P mobilization. This mainly involves proton efflux to acidify the rhizosphere, exudation of carboxylates to mobilize sparingly soluble P via chelation and ligand exchange, and the secretion of phosphatases to mineralize organic P forms in the soil (Po) (Pang et al., 2010; Zhang et al., 2010; Lynch, 2011; Bayuelo-Jiménez and Ochoa-Cadavid, 2014). For details, readers are referred to recent reviews by Lambers et al. (2006) and Richardson et al. (2011).

These strategies facilitate the mobilization of P from these non-labile pools, and thereby P availability has been enhanced over a large timescale in weathered soils with the evolution of these strategies (Lambers et al., 2008). These evolved strategies induce feedback processes between plants and soils, which are relevant to the photosynthetically fixed C and its allocation (Buendía et al., 2014). Increased C fixation and more below-ground investments promote P-enhancing processes in the soil (DeLucia et al., 1997; Allen et al., 2003).

Thus, an important consideration here is that elevated CO$_2$ will generally increase the C allocations to roots and the increase in root C will stimulate root growth (Rogers et al., 1992, 1994; Li et al., 2012) and increase exudate secretions from the roots. This, in turn, will influence conditions in the rhizosphere, which is the interface between plant roots and soil (Paterson et al., 1997; Haase et al., 2008; Drigo et al., 2013). The changes in rhizosphere environment are likely to affect P acquisition by plants. Questions therefore arise as to whether plant P demand on the one hand and P acquisition on the other will be affected more by the increase of atmospheric CO$_2$ concentrations. Understanding this supply–demand balance for labile soil P will be important for developing P management strategies in agricultural systems to cope with increasing atmospheric CO$_2$ concentrations.

In this review, we examine the current state of knowledge with respect to plant P demand under elevated CO$_2$ and then focus on the associated mechanisms of P acquisition. This includes changes in root morphology, root exudates and relevant rhizosphere processes that may affect P mobilization and transformations in soils. These possible effects of elevated CO$_2$ are summarized in Fig. 1, which provides the framework of this review. The need for further research into P functioning in ecosystems in an elevated CO$_2$ environment is then highlighted.

**PLANT P DEMANDS UNDER ELEVATED CO$_2$**

Plant P requirement can be divided into the need for external soil P and the need for internal P within the plant tissues. The external P requirement is the available P in soil that is required to produce 90 % of the maximum plant yield (Sattar et al., 2011). Similarly, the internal P requirement is the P concentration in the plant to achieve 90 % of maximum yield (Loneragan and Asher, 1967; Sattar et al., 2011). The external and internal P requirements therefore represent the P-acquisition efficiency and P-use efficiency for yield production, respectively (Föhse et al., 1988; Veneklaas et al., 2012).

The external P requirement is likely to increase with increased plant growth under elevated CO$_2$ (Table 1). However, the extent of this requirement will depend on the plant species. In general, the growth response to elevated CO$_2$ is greater in C$_3$ species than C$_4$ species, as the CO$_2$ saturation point in C$_3$ species (50–150 mg L$^{-1}$ CO$_2$) is higher than C$_4$ species (1–10 mg L$^{-1}$ CO$_2$), and the photosynthetic capability can be greatly enhanced in C$_3$ species under elevated CO$_2$ (Wand et al., 1999; Lee, 2011). For example, the yield of wheat (C$_3$) increased by 31 % with elevated CO$_2$ at 500–700 μL L$^{-1}$ in a Free Air CO$_2$ Enrichment (FACE) facility (Mauney et al., 1999; Arabo, 2001; Jablonski et al., 2002), whereas sorghum (C$_4$) yield was not increased in the same environment (Otman et al., 2001). Within C$_3$ species, legume species display larger growth responses to elevated CO$_2$ (600–700 μL L$^{-1}$) than non-legume species due to the enhanced N$_2$ fixation (Stöcklin and Körner, 1999; Joel et al., 2001; Cernusak et al., 2011). Interestingly, a meta-analysis showed that trees had a greater response to elevated CO$_2$ (475–600 μL L$^{-1}$) than legumes and C$_3$ grasses in dry matter production (Ainsworth and Long, 2005). As the plant P demand generally increases along with growth stimulation by elevated CO$_2$ (Edwards et al., 2005; Gentile et al., 2012; Zhang et al., 2014), this larger growth response by trees than C$_3$ species and legumes grown under elevated CO$_2$ suggests that trees would exhibit a higher P demand under elevated CO$_2$.

The critical levels for the external P requirements have not been established under elevated CO$_2$. However, several studies with different plant species found that the external P requirements were greater under elevated than under ambient CO$_2$ (Conroy et al., 1990; Barrett and Gifford, 1995; Lewis et al., 2010; Jin et al., 2012). This can be seen in Table 1 where most species increased P uptake by shoots in response to elevated CO$_2$ concentrations. This was the case with the growth of cotton wood (Populus deltoides) in a sand–gravel root medium with P supplied at six concentrations from 0-004 to 0-5 μM (Lewis et al., 2010). A similar situation was reported for chickpea (Cicer arietinum) and field pea (Pisum sativum) grown in a P-deficient Vertisol with increasing added P from 0 to 16 mg P kg$^{-1}$ soil (Jin et al., 2012). In these studies, maximum growth to added P was not achieved. Nevertheless, they showed a similar result that the growth responses to elevated CO$_2$ (550–700 μL L$^{-1}$) were more pronounced under P-sufficient than P-deficient conditions.

Elevated CO$_2$ is likely to affect the internal P requirement of plants because elevated CO$_2$ alters P utilization within plant tissues (Niu et al., 2013). Although the internal P in many species has been investigated under ambient CO$_2$ environments
Elevated CO₂

Shoot growth

Shoot residues

Root residues

Mycorrhizal fungi

P availability

Carbon allocation

P uptake

P demands

P-associated metabolism

Morphology changed

Root biomass

Root length

Density & length of root hair

Microbial activity

Microbial structure?

Microbial function?

Exudates

Hydrolysing

Phosphatases

Organic P

Labile P

Inorganic P

AI-P, Fe-P, Ca-P etc.

Mineralization

Immobilization

Rhizosphere pH

Composition & quantity?

Carboxylates

Phenolics

Phosphates

Future P management

FIG. 1. Proposed mechanisms by which elevated CO₂ impacts plant P nutrition. † indicates an increase and “?” indicates an unknown effect.

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<th>Table 1. Plant P requirement under elevated CO₂</th>
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[P], P concentration; †, increase; ‡, decrease.
Elevated CO₂. More than 50% of P in plants is redistributed to assimilation. Tissue is used for photosynthesis-associated metabolisms and efficiency would increase, as a greater proportion of P in plant roots is transformed into Po for the synthesis of Rubisco because Po is the enzyme Rubisco which functions in photosynthesis and so contributes to plant growth (Elser et al., 2010; Reef et al., 2010).

Elevated CO₂ is likely to affect the transformation of P from inorganic to organic form in plant tissue, thereby mediating P-use efficiency. The increase in photosynthetic rate and plant growth under elevated CO₂ is linked to the concentration of the enzyme Rubisco. P is stored in the vacuole and acts as a buffer to meet the P demands from the cytoplasm (Veneklaas et al., 2012). The largest Po pool in plant is the nucleic acid pool, which accounts for 40–60% of the total Po pool. In this pool, RNA is the dominant component, with ribosomal RNA (rRNA) making up more than 80% of the total (Kanda et al., 1994). The rRNA is required for synthesizing proteins such as the enzyme Rubisco which functions in photosynthesis and so contributes to plant growth (Elser et al., 2010; Reef et al., 2010).

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Internal redistribution of P within the plant may be altered by elevated CO₂. More than 50% of P in plants is redistributed to new growing points, especially during later growth stages and under P-deficient conditions (Aerts, 1996). Growth rates decline during the reproductive stage, including root expansion, and so P uptake by root systems decrease. Thus, uptake-terminated P supply is shifted to remobilization-dominated P supply. However, when plants are exposed to elevated CO₂, the growth rate of the shoots increases together with an increase in the carbon allocation to roots, and this generally increases the root-to-shoot C ratio (Ainsworth et al., 2003; Jin et al., 2012).

How these changes affect P redistribution in plants is not known. In addition, the extent of the translocation of P to developing grain is not known. However, it is likely that increasing the grain yield response under elevated CO₂ will result in increased P exports in the grain from the field, given the high content of phytate P in cereal grain (Buddrick et al., 2014).

THE EFFECT OF ELEVATED CO₂ ON PLANT STRATEGIES TO ACQUIRE P

Current crop production in P-deficient soils is heavily reliant on the application of P fertilizers. However, more intensive P fertilization is likely to become problematic in the long term, to provide for the increasing P demands of crops under elevated CO₂, because reserves of phosphate ore deposits are finite (Lynch, 2011). There are also concerns about the environmental impact resulting from intensive P fertilization. Thus, it is increasingly important to improve plant P acquisition and P-use efficiency under elevated CO₂.

Elevated CO₂ is likely to affect the P acquisition strategies in several ways. The increase in C assimilation in plants grown under elevated CO₂ is likely to lead to a considerable response in root growth, including changes in root architecture and morphology that will affect P acquisition from soil profiles. Second, the composition and quantity of root exudates are likely to alter under elevated CO₂ and hence these will change rhizosphere properties such as pH, Eh and the capacity for chelation and ligand exchange, which in turn will affect P availability. Third, these root exudates may also modify the association between microorganisms and P transformations. These impacts on P acquisition strategies under elevated CO₂ are addressed in the following sections.

Root morphology traits under elevated CO₂ in relation to P acquisition

As P is an immobile nutrient in soil, increases in root length and root branching under elevated CO₂ may increase the plant’s capacity to acquire P from the soil. The effect of a larger root system is shown by the work of Hammond et al. (2009). They reported that P uptake in Oryza sativa and Brassica oleracea genotypes under low P supply was correlated with lateral root growth rate, lateral root length, the number of lateral roots and root surface area. In addition, the root hairs also contributed to P acquisition with direct evidence coming from studies with mutant plants with no root hairs (Bates and Lynch, 2000), and from the comparison of species and genotypes that have contrasting length and density of root hairs (Richardson et al., 2011). These changes in root morphology that develop in response to P deficiency are important for P-acquisition efficiency by plants (Lambers et al., 2006; Pang et al., 2010).

Root morphology will probably change in response to elevated CO₂ and this will alter the P-acquisition efficiency. The increase in photosynthetic C allocation to roots under elevated CO₂ results in stimulation of root growth more than the growth of other plant organs (Norby et al., 1992; Benlloch-Gonzalez et al., 2014). The elevated CO₂-mediated increase in root growth will bring about increases in root length, root number,
root diameter and root branching. Yang et al. (2007) showed that compared with ambient CO2 (350 μL L⁻¹), 550 μL L⁻¹ increased root biomass by 45%, root volume by 44%, number of adventitious roots by 31% and overall root length by 37% when rice plants were grown in a Stagnic Anthrosol soil. A greater number of root clusters and a higher percentage of lateral roots were also observed in white lupin (Lupinus albus) grown under elevated CO2 (Watt and Evans, 1999; Campbell and Sage, 2002). Similar trends were found in chickpea, soybean, field pea, wheat, sorghum and cotton (Del Castillo et al., 1989; Rogers et al., 1992, 1994; Jin et al., 2013, 2015). These changes in root morphology result in an increase in the spread of roots through the root zone, which should lead to increases in nutrient uptake (Baker et al., 1990; Idso and Kimball, 1991, 1992; Rogers et al., 1992). A similar result was found by Jin et al. (2012), who reported a significant positive relationship between root length and P uptake under both ambient CO2 and elevated CO2. The longer roots under elevated CO2 in that study resulted in greater P acquisition. Thus, it appears that root growth positively responds to elevated CO2, enabling the roots to explore a larger volume of soil, and this will increase the plant’s ability to take up nutrients (Nie et al., 2013), especially immobile phosphate ions.

The response of root morphology to elevated CO2 and the impact on P acquisition are fundamentally regulated at the genetic level. Ainsworth et al. (2006) reported that there were 327 independent genes that were CO2-responsive when soybean plants were exposed to elevated CO2, while Raghothama (1999) reported that there were more than 100 genes involved in plant response to elevated CO2.

Auxin genes including auxin-responsive promoters (Chandler, 2009) and auxin transport genes (Santelia et al., 2005) are thought to be the most responsive genes to elevated CO2 and external P status. Auxins are hormonal compounds that regulate plant growth processes, such as the initiation and elongation of root hairs (Pitts et al., 1998; Schiefelbein, 2000).

Nie et al. (2011) found that elevated CO2 resulted in the expression of auxin-specific genes, which were likely to enhance the growth of root hairs in Arabidopsis. On the other hand, auxin genes that are responsive to P availability are involved in the regulation of the P starvation response in roots (Narcy et al., 2005; Jain et al., 2007). The expression of auxin-responsive genes responds to P deficiency by stimulating pericycle cells to produce lateral roots (López-Bucio et al., 2005). Pérez-Torres et al. (2008) further showed that P deficiency increased the expression of the auxin receptor TRANSPORT INHIBITOR RESPONSE 1 (TIR1), which enhanced the sensitivity of auxins to increase the emergence of lateral roots. Therefore, the expression of these plant genes within a given environment triggers molecular, physiological and cellular processes that modify root architecture (Gilroy and Jones, 2000; Niu et al., 2013b). Further investigation of these genetic factors that mediate root development will be required to reveal the molecular mechanisms by which the plant adapts to P deficiency and to elevated CO2 environments. Specifically, the quantitative relationship between auxins and pericycle cell division leading to the development of new roots, and the elevated CO2/P supply responsive molecular pathways that regulate the expression of auxin-responsive genes warrant future studies.

Rhizosphere processes in response to elevated CO2 and their impacts on P availability

The effect of elevated CO2 on rhizosphere properties is likely to impact on the ability of plants to acquire P from the soil. Elevated CO2 is likely to increase C flow from plant to soil by increasing the release of root exudates (Lin et al., 2000; Song et al., 2014). These exudates contain functional molecules which facilitate an increase in rhizosphere P solubility, and hence improve P nutrition to plants (Richardson et al., 2009). Furthermore, root exudates are responsible for changes of rhizosphere pH and increases in microbial activity (Shen et al., 2011). These effects of elevated CO2 can change P availability in the rhizosphere and consequently influence plant P acquisition (Norby et al., 2001; de Graaff et al., 2006).

Root exudates

Exudates released from roots into the rhizosphere can affect the availability of soil P to plants (Randall et al., 2001; Betencourt et al., 2012). Low-molecular-weight carboxylates present in root exudates have been considered to be Pi-mobilizing agents (Johansson et al., 2009). The effectiveness of these carboxylates to mobilize P depends largely on carboxyl (-COOH) and hydroxyl (-OH) functional groups in these molecules. Citrate (tricarboxylic acid, TCA) exhibits the greatest ability to desorb P, followed by oxalate (dicarboxylic acid), while malate, malonate and tartarate are moderately effective (Bolan et al., 1994; Jones, 1998; Jones et al., 2009; Richardson et al., 2009). Citrate is particularly effective at mobilizing P from Fe-phosphates and Al-phosphates in acid soils (Bolan et al., 1994) and Ca-phosphates in calcareous soils, or from rock phosphate fertilizer (Dinkelaker et al., 1989).

The mechanism by which the carboxylates in root exudates affect soil P mobilization under elevated CO2 is not known. Shen et al. (2011) suggested that P is mobilized by desorbing and chelating P from Al-P and Fe-P complexes and from other non-labile pools. However, the extent that elevated CO2 increases P desorption depends on whether elevated CO2 stimulates the release of those carboxylates that are effective in mobilizing Pi.

Significant volumes of root exudates have been measured following elevated CO2 exposure (Cheng and Johnson, 1998; van Ginkel et al., 2000; Allard et al., 2006). For example, after 34 weeks of growth under elevated CO2, the exudation of soluble C compounds from roots of short-leaf pine increased by 50% (Norby et al., 1987). Similarly, the release of low-molecular-weight organic compounds increased by 120–160% and amino acids increased by 250% when Pinus sylvestris was grown for 5 weeks in a nutrient solution under elevated CO2 (700 μL L⁻¹) in comparison with ambient CO2 (350 μL L⁻¹) (Johansson et al., 2009). Haase et al. (2007) also found that the release of malate, which is the major organic acid in the exudates from Phaseolus vulgaris, increased by 177% after the plants were exposed to elevated CO2 (800 μL L⁻¹) for 18 d. The increase of these organic compounds is likely to mobilize P in the rhizosphere but to date the mobilization of P in the rhizosphere has not been assessed quantitatively.

There are even fewer studies that have investigated the composition of root exudates in response to elevated CO2.
One investigation was carried out by Watt and Evans (1999) to measure the composition of organic acid anions including citrate, oxalate, α-ketoglutarate, malate, succinate, pyruvate and fumarate from white lupins (Lupinus albus) grown under elevated CO2 (700 µL L−1). No significant effect of elevated CO2 was observed on the composition of these anions during 4 weeks of hydroponic culture. It may be that the release of organic acid anions in response to elevated CO2 varies with plant species, growth stage and conditions. Further research to screen P-efficient plant species for their efflux of organic acid anions in response to elevated CO2 is recommended. Such work would improve our understanding of the adaptive mechanism of plant species to P deficiency under elevated CO2.

How the P-mobilizing carboxylates in root exudates respond to elevated CO2 needs to be interpreted at the metabolic level. The carboxylates released by roots are thought to be the products from the glycolytic pathway and the TCA cycle, which occur in roots with the involvement of the phosphoenolpyruvate carboxylase (PEPc) enzyme (Johnson et al., 1996; Massonneau et al., 2001). Malate, for example, is generated from the carboxylation of PEP to produce the glycolytic end-product PEPc (Cramer et al., 2005). It has been experimentally shown using 14C labelling that an increase in C supply was accompanied by the increased specific activity of PEPc and exudation of organic acid anions (Johnson et al., 1996; Ulhe-Storey et al., 2003). Interestingly, elevated CO2 increased the transcription levels of genes encoding enzymes of glycolysis and the TCA cycle. Under elevated CO2, the TCA cycle accelerated with higher substrate availability (Ainsworth et al., 2006). Under P deficiency, PEPc activity was also increased in plants such as chickpea and oilseed rape (Hoffland et al., 1992; Moraes and Plaxton, 2000). Thus, the regulation of the synthesis-associated genes for these enzymes is essential for the production of P-mobilizing carboxylates in the glycolytic pathway and TCA cycle under elevated CO2.

The phenolics are a group of secondary metabolites that mobilize P in soil, and are likely to be influenced by elevated CO2 as well. A study on the biosynthesis of phenolics showed that the activity of the principal phenolic biosynthetic enzyme in Senecio vulgaris increased under elevated CO2 (Hartley et al., 2000). Based on a 2-year field experiment in open-top chambers (375 vs. 550 µL L−1), Goufo et al. (2014) reported that the concentration of most phenolic compounds, such as apigenin, sinapic acid, chlorogenic acid, caffeine acid, protocatechuic acid, tricin and apigenin 7-O-glucoside, increased significantly in the rhizosphere of mature rice under elevated CO2. These results indicate that elevated CO2 enhances the release of phenolics from root systems, and these may in turn increase the P availability in soils. The role of phenolics in mobilizing P has been illustrated in calcareous and acid soils. Hu et al. (2005a, b) showed that phenolics such as caffeic, protocatechuic, p-coumaric and vanillaic acid exhibit varying capabilities in P mobilization. Their effectiveness depends on the number of phenolic hydroxyl groups that phenolics have and the position of the carboxyl group on the benzoic ring. Furthermore, isoflavonoids are a class of phenolic compounds that are increasingly exuded from white lupin roots under P deficiency. These isoflavonoids include genistein and hydroxygenistein and their corresponding mono- and di-glucoside conjugates (Weisskopf et al., 2006). These isoflavonoids are mainly exuded in juvenile and immature cluster roots, and are thought to inhibit the soil microflora from breaking down P-mobilizing citrate in the exudates (Weisskopf et al., 2006).

**Rhizosphere pH**

Soil pH can greatly influence the solubility of P in soils (Shen et al., 2011). In acid soils where the concentrations of trivalent Fe and Al are high, labile Pi in soil solution is easily precipitated as Fe- and Al-phosphates or sorbed onto Fe- and Al-(hydroxides. In contrast, in alkaline soils where Ca is the major cation, Pi is predominantly precipitated as Ca-phosphates (Richardson et al., 2009). Thus, soil pH from 6.0 to 7.0 provides optimal conditions for P solubility (Hinsinger, 2001). Given this relationship between soil pH and P availability, any process that alters soil pH will influence P availability in the soil solution.

There are several ways that elevated CO2 is able to change P availability by modifying the rhizosphere pH. The first is that elevated CO2 may change the quantity of organic acid anions and associated protons released in exudates from plant roots, leading to pH changes in the rhizosphere (Guo et al., 2012). Organic acid anions have often been associated with the release of protons as a source of rhizosphere acidification (Hoffland et al., 1989; Hinsinger et al., 2003). For example, the release of citrate from cluster roots of white lupin was associated with strong rhizosphere acidification (Neumann and Römheld, 1999), which suggests that H+ ions released to accompany the efflux of citrate were a major component of the observed acidification of the rhizosphere. As elevated CO2 is likely to increase the exudation of organic acid anions, the H+ extrusion accompanying this exudation would lower pH and thereby enhance P mobilization in alkaline soils rather than acidic soils (Lynch, 2011; Bayuelo-Jímenez and Ochoa-Cadavid, 2014).

The second way that elevated CO2 might impact on rhizosphere pH results from the large amount of CO2 derived from the respiration of the root and the microbes in the rhizosphere under elevated CO2. The increased activities of rhizosphere microorganisms (Jin et al., 2014) under elevated CO2 are likely to increase CO2 concentration in soil (Matamala and Schlesinger, 2000; Carrillo et al., 2014) and this CO2 will dissolve in soil H2O to form H2CO3. As a result, the pH in the rhizosphere is likely to decrease. However, this scenario in terms of rhizospheric pH may be marginal, because gaseous CO2 diffuses much faster than H2CO3 in solution (Anoua et al., 1997; Hinsinger et al., 2003), and only neutral to alkaline soils can respond to the change in soil CO2 concentrations because H2CO3 with its first pK of 6.36 remains undissociated at low pH (Lindsay, 1979).

The third way that elevated CO2 impacts on rhizosphere pH involves N2-fixing legumes. When legumes fix N2, the plants take up more cations than anions and thus extrude H+ ions from their roots to maintain charge balance (Tang et al., 1997). Given that elevated CO2 stimulates nodulation and N2-fixation (Prévost et al., 2010), legume plants are likely to extrude more H+ ions and decrease the rhizosphere pH, relative to non-legumes, under elevated CO2. It would be interesting to determine the pH variation in the rhizosphere of legumes and non-legumes in response to elevated CO2. Changes in
rhizosphere pH in response to elevated CO₂ would depend on the balance between the cation–anion exchange across the plasma membranes of the root cells of the plants being compared.

**Rhizosphere microorganisms**

Elevated CO₂ directly influences the density, diversity and functioning of the rhizosphere microbial communities (Paterson et al., 1996; Hodge and Millard, 1998; Haase et al., 2008). Drissner et al. (2007) found a 48-1 % increase in soil microbial biomass and 12.5 % increase in the Shannon index (species diversity in a community) of bacterial community structure after *Trifolium repens* L. and *Lolium perenne* L. had grown under elevated CO₂ in a FACE facility for 9 years. Similarly, microbial growth rate per unit soil mass in the rhizosphere of *Populus deltoids* was up to 58 % higher under elevated CO₂ than under ambient CO₂ (Blagodatskaya et al., 2010). In addition, microbial respiration and the metabolic quotient of microbes in the rhizosphere of wheat increased significantly under elevated CO₂ (Jin et al., 2014).

Elevated CO₂ is able to specifically affect the abundance of some microbial genera, which may directly facilitate P solubilization in the rhizosphere. Drigo et al. (2009) found that the abundance of *Pseudomonas* bacteria in the rhizosphere increased under elevated CO₂, with active populations of *P. aeruginosa*, *P. fluorescens*, *P. trivialis* and *P. putida* being detected. Both *P. fluorescens* and *P. putida* are considered to be P-solubilizing microorganisms that produce metabolites that solubilize sparingly soluble inorganic P compounds to release phosphate ions (Egamberdiyeva and Höflich, 2003; Krey et al., 2013). Similarly, P-solubilizing bacteria associated with proteoid roots of *Telephoea speciosissima* are able to release P from calcium phosphate (Wenzel et al., 1994). This suggests that elevated CO₂ is likely to benefit these P-solubilizing microorganisms. However, the magnitude of this effect depends on the P compounds in soils, and the plant species, which in turn will determine the abundance of the P-solubilizing microbial species in these rhizospheres (Wenzel et al., 1994).

Arbuscular mycorrhizal fungi (AMF) are likely to be stimulated by elevated CO₂, which will assist P acquisition by the host plant. In this symbiotic relationship, AMF provide their host plants with mineral nutrients, such as P, in exchange for carbohydrates supplied to the AMF (Kiers et al., 2011). This two-way transfer of resources is certainly affected by elevated CO₂, because elevated CO₂ increases C allocation to the roots of the host plant (Gamper et al., 2004). Studies have found that the AMF hyphal network is enlarged by elevated CO₂, resulting in nutrient absorption being significantly increased (Gamper et al., 2004; Staddon et al., 2004). With a meta-analysis, Treseder (2004) also found that the abundance of AMF increased relative to root length under elevated CO₂. Furthermore, shifts in active AMF species under elevated CO₂ were convincingly confirmed using stable isotope (³¹C) probing and subsequent real-time PCR techniques (Drigo et al., 2010). The increase in symbiotic activity between AMF and plants under elevated CO₂ leads to an expectation that mycorrhizal plant species will adapt better to P-deficient soils compared with non-mycorrhizal species in the elevated CO₂ environment.

On the other hand, it cannot be ignored that elevated CO₂-induced increases in the microbial biomass and activity will mean that these microbes may compete for more P, resulting in P immobilization. The P immobilized by microbes is not negligible, because soil microorganisms constitute a small but significant component of total soil P, typically accounting for around 2–10 % (Achat et al., 2010; Richardson and Simpson, 2011). A recent study found that microbial P in the rhizosphere increased by more than 20 % when wheat plants were grown under elevated CO₂, indicating microbes were the main source of P immobilization occurring under elevated CO₂ (Jin et al., 2014). The microbial C/P ratio did not change under elevated CO₂ in that study, indicating the increase of microbial P was attributed to the change of microbial biomass C, rather than any change in P composition in microorganisms. This indicates the importance of microbial populations in enhanced P immobilization in the rhizosphere.

**Rhizosphere enzymes**

The change in rhizosphere enzyme activity in response to elevated CO₂ is likely to affect P mineralization in the rhizosphere. The activities of many enzymes were stimulated by root proliferation under elevated CO₂ (Haase et al., 2008) including invertase (48 %), xylanase (23 %), urease (24 %), protease (40 %) and alkaline phosphomonoesterase (54 %) (Drissner et al., 2007). Most of these enzymes are involved in nutrient transformation and include phosphatases, which are enzymes that catalyse the transformation of Po to Pi. A study at a tundra site showed that phosphatase activity on the root surface of *Eriophorum vaginatum* was 254 % higher under elevated CO₂ than under ambient CO₂, and this contributed to a more than 40 % increase in the annual P release within tussocks (Moorhead and Linkins, 1997). On the other hand, elevated CO₂ did not alter either the acid phosphatase or the alkaline phosphatase activity in the rhizosphere of chickpea or field pea grown in a P-deficient Vertisol (Jin et al., 2012). Furthermore, Haase et al. (2008) found that the activity of phosphatases in the rhizosphere of *Phaseolus vulgaris* L. decreased under elevated CO₂. The discrepancy between the studies may be explained by differences in organic matter content of the soils. The P availability in soils with high organic matter (>117 g C kg⁻¹ soil) in the arctic tundra ecosystem is likely to depend on phosphatase activity (Moorhead and Linkins, 1997), while the content of organic matter in the soils used in the latter studies were less than 1 g C kg⁻¹ soil.

Understanding the mechanisms by which elevated CO₂ affects phosphatase enzymes remains a challenge. Phosphatase enzymes are either of plant or microbial origin. A wide range of plant species secrete phosphatases into their rhizosphere. These plant species include sorghum (*Sorghum bicolor*), cowpea (*Vigna unguiculata*) and mung bean (*Vigna radiata*) (Tarafdar and Claassen, 2001; Lambers et al., 2006). Similarly, soil microorganisms such as *Aspergillus* sp. and mycorrhizas produce phosphatases (Tarafdar, 1995). In this respect, the question is raised as to how elevated CO₂ affects (1) the population of phosphatase-producing microbes in the rhizosphere and (2) the activity of phosphatases exuded from the roots of plant species, and (3) what each of these contributes to P...
mineralization. However, it is necessary to quantitatively identify the origin of phosphatases before investigating the elevated CO2 effect on them.

More recently, the link between phosphatase activity and photosynthetic supply has been established. Spohn and Kuziyakov (2013) developed an approach to studying the distribution of phosphatases and photosynthetic C supply using 14C imaging and soil zymography, which provides in situ mapping of the two-dimensional distribution of enzyme activity in soil. This approach allows us to understand the relationship between elevated CO2-driven changes in the allocation of below-ground biochemical reactions that become dominant in P transformations. Radioisotopes 32P or 33P have been used to investigate the P dynamics in soil (McLaughlin et al., 1988; Daroub et al., 2000; Vu et al., 2010; Noack et al., 2014). Studies reported that up to 25% of added 32P in soil was recovered in soil microorganisms (Oberson et al., 2001), and 20–27% of added 33P in Po fractions (Bühler et al., 2003; Bünemann et al., 2004), highlighting the importance of biological transformation of P in soil. In addition, a new precipitation approach using 33P nuclear magnetic resonance (NMR) imaging is able to characterize Po molecules in soils (Vestergren et al., 2012). The approach would be useful in understanding the P fluxes that occur in the rhizosphere in response to elevated CO2.

THE IMPACT OF ELEVATED CO2 ON P MINERALIZATION OF PLANT RESIDUES

The change in quality of plant residues under elevated CO2 is likely to influence the P cycling in ecosystems. A fundamental change of quality in residues produced in the elevated CO2 environment will be the reduction in N concentration in the residues, particularly of non-legumes (Butterly et al., 2015). Cotrufo et al. (2005) provided experimental data showing that N concentrations in plant tissues generated under elevated CO2 declined by an average of 14%. Thus, with the increased C/N ratio, the decomposition rate of plant residue may be limited by the lower N concentrations, and lowered further in N-deficient soils (Viswanath et al., 2010). Similarly, the increase in C/P ratio may occur under elevated CO2, as elevated CO2 leads to a decrease of P concentration in some species such as Glycine max, Eucalyptus grandis and Agrostis capillaris (Conroy et al., 1992; Newbery et al., 1995; Gifford et al., 2000). As a consequence, the high C/P may further inhibit the decomposition process of plant residues, combined with N limitation. The slow decomposition will mean that the residues returned to soil over a longer time scale result in a reduced rate of P transformation from organic to inorganic forms, which will lower the P supply to plants over time. Whether this scenario occurs in the future depends on how P-acquisition strategies evolve on the ability of plant regulating root exudates, altering microbial functions, and thereby favouring P mineralization.

Identifying the magnitude of the P supply from decomposing residues is a challenge. It has been reported that about 40–60% of P in residues is water-soluble and can be mineralized into soils at initial stages of decomposition (Ha et al., 2008). However, if plant residues with a C/P ratio of more than 300 are added to soils, then a net immobilization of P is likely to occur (Iyamuremye et al., 1996; Ha et al., 2008). Under elevated CO2, it is not certain whether the water-soluble P composition varies in residues, and whether the increased C/P ratio exceeds
the threshold. These will be associated with their C chemistry, which determines the form of P incorporated in residues. In addition, the N/P ratio in residues is a significant factor which will determine whether mineralization or immobilization of P will occur when the residue is incorporated into soil (Kwabiah et al., 2003). This raises the question of which nutrient (N or P) becomes the dominant factor limiting P supply during the decomposition of residues in the elevated CO2 environment. This question will require answers from long-term investigations.

**FUTURE PERSPECTIVES**

Phosphorus nutrition in plants growing in the terrestrial domain is likely to undergo considerable change under elevated CO2. Although there is limited information on the difference in the impact of elevated CO2 on P nutrition between agricultural and natural ecosystems, it is likely that differences between these systems will occur. The P acquisition of plants originated from P fertilizer would change considerably in the agricultural ecosystem, while the internal and external P utilization would tend to be intensively improved in the natural ecosystem.

It is likely that increases in P fertilization rates will be required in agricultural systems with increases in the concentrations of atmospheric CO2. More P would be needed to meet the increased demand for P by crop plants resulting from the ‘CO2 fertilization effect’ on crop growth. The required increase in P fertilizer rates will depend on the balance between extra P demand by crop species under elevated CO2, and the increased capacity of roots to mobilize soil P and to forage for the labile P in soil. Nevertheless, for crop plants in general, the evidence suggests that increased P fertilization will be required to improve the adaptability of cropping systems to increasing atmospheric CO2 concentrations. This is a concern as the need for more P fertilizer inputs raises questions about long-term sustainability and food security, and environmental impact. Supplies of P rock for manufacturing P fertilizer are finite and sustainably and food security, and environmental impact. Supplies of P rock for manufacturing P fertilizer are finite and have learnt how the loss of P from agricultural systems can impact negatively on terrestrial water bodies.

Plants in natural systems will continue to adapt to changing environmental conditions. Plants have adapted to low-P soils by developing P acquisition strategies, and this will continue. There will be increasing selection pressure for P-acquisition efficiency, by plants and plant–microbe associations in the high-C environment. They will utilize and exploit the increased C flow to their roots to more efficiently mobilize and/or forage for labile P forms in the soil. The mechanisms for this selection might include the development of longer roots, more lateral roots and root hairs, changes in the quantity and composition of root exudates, and changes in the activities and/or functions of microbes and plant–microbe associations. These adaptation strategies will enable plants to compete for P in the elevated CO2 environment.

Optimizing P management for crop plants in the future requires a more detailed understanding of plant–soil interactions in response to elevated CO2 (see Fig. 1). This includes understanding the biochemical processes as to how elevated CO2 mediates C allocation to root development, root metabolism and the release of root exudates in the rhizosphere. Improved understanding is also needed on how these processes affect microorganisms in the rhizosphere, because these microorganisms can impact significantly on P availability.

A range of experimental approaches are suggested for further research. The first is to undertake geno-to-pheno investigations from the CO2-induced gene expression in the plants and how this expression influences root architecture formation and root exudate metabolism, as both will affect P acquisition. A second approach would be to use photosynthetic 13C tracing studies to identify soil microbial communities that are responding to elevated CO2 and are involved in either immobilization or mineralization of P in the rhizosphere. A third approach would be to identify P-containing molecules in the rhizosphere using NMR to determine the quantity and the composition of these molecules during the P transformations under elevated CO2. These studies need to be undertaken with different plant species in different soils.

**ACKNOWLEDGEMENT**

This research was supported by an Australian Research Council Linkage Project (LP100200757).

**LITERATURE CITED**


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