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REGULAR ARTICLE

Long-term impact of elevated CO_2 on phosphorus fractions varies in three contrasting cropping soils

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Abstract
Background and aim The long-term effect of elevated CO_2 (eCO_2) on P biogeochemistry in farming systems is largely unknown. This study compared the effects of eCO_2 on P fractions in three contrasting soils after growing crops for seven years.
Methods An experiment of free-air-CO_2-enrichment (FACE) was conducted with a rotation of wheat, field pea and canola grown in intact cores of Chromosol, Vertosol and Calcarosol under ambient CO_2 (aCO_2) (390 ± 10 ppm) or eCO_2 (550 ± 30 ppm). Crop P removal, soil P fractions and biochemical properties were determined.
Results Elevated CO_2 resulted in extra 134, 91 and 93 mg P $core^{-1}$ removed as grains, compared to aCO_2, for Chromosol, Vertosol and Calcarosol, respectively. It decreased the concentration of $NaHCO_3$-extractable inorganic P (by 17–36%), and decreased NaOH-extractable inorganic P by 24% in Chromosol, and 77% in Vertosol but did not affect it in Calcarosol. Elevated CO_2 also decreased NaOH-extractable organic P by 20, 12 and 7 mg kg^{-1} in the three soils, respectively. Furthermore, eCO_2 decreased soil organic carbon (by 8.2%) and increased microbial biomass carbon and respiration in Chromosol but not in other two soils.
Conclusion Long-term eCO_2 favoured microbial mineralization of organic P in Chromosol and chemical mobilization of non-labile inorganic P in all three soils.

Keywords C/P ratio · Face · High atmospheric CO_2 · Microbial processes · P fractionation · P removal · Soil organic C

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Introduction

Phosphorus (P) cycling in agricultural cropping systems is likely to be impacted by the elevated atmospheric CO_2 concentration (Ochoa-Hueso et al. 2017). Under elevated CO_2 (eCO_2) environments, the demand for P increases in many plant species (Bhattacharyya et al. 2014; Jin et al. 2015). For example, the relative growth response of chickpea (*Cicer arietinum* L.) and field pea (*Pisum sativum* L.) to eCO_2 (550 ppm) was more pronounced under P-sufficient than P-deficient conditions (Jin et al. 2012). Moreover, total P uptake of the plant was greater under eCO_2 compared with ambient CO_2 (aCO_2) although plant P concentration was generally lower due to a dilution effect (Jin et al. 2012, 2013). As grain yields tend to increase and more P contained within the grain exported under eCO_2, the net amount of P in the soil is likely to decrease accordingly in the long term if the soil P is not fully replenished by P fertilizers. Knowledge of the relationship between soil P status and

crop removal is essential for development of long-term fertilization strategies to sustain crop productivity in the future eCO_2 environments.

Previous studies have shown that eCO_2 can increase or decrease soil P status. For example, labile soil P increased by approximately 23%,while the recalcitrant P was depleted by 27% after six tree species had been exposed to eCO_2 (700 ppm) for five years (Huang et al. 2014). Similarly, compared with aCO_2, 200 ppm higher CO_2 concentration increased soil $NaHCO_3$-extractable P by 14% when wheat was grown for one season in a loamy soil in a Free Air Carbon Dioxide Enrichment (FACE) system (Zhang et al. 2014). However, a decrease in soil P availability was found after *Deschampsia flexuosa* and *Calluna vulgaris* were exposed to 1 year of eCO_2 in a heathland FACE facility without any P input (Andresen et al. 2010). These inconsistent findings about how eCO_2 affects soil P availability is likely to reflect the various growth responses to eCO_2 among plant species, and different soil physical and biological properties and P fertilizer inputs, all of which influence soil P transformations (Lukac et al. 2010). Little information is available on the long-term impact of eCO_2 on P pools particularly in Australian agricultural soils which are highly weathered and naturally deficient in soil P availability (Lambers et al. 2010; Rossel and Bui 2016).

The dynamics that occur between various P pools in soils is complex. Phosphorus exists in inorganic mineral forms (Pi) and in organic forms (Po) derived from both vegetation and microbial biomass turnover (Turner et al. 2005; Khan et al. 2008). The Pi in soils composes of a continuum of fractions in equilibrium with each other, which differ in their availability to plants. These equilibria are likely regulated by eCO_2-induced changes in chemical processes such as pH. On the other hand, eCO_2 may affect Po fractions through biological immobilization or mineralization due to microbial turnover and release of phosphatase (Richardson and Simpson 2011). Thus, which P fraction eCO_2 may greatly affect, to large extent, depends on the accessibility of these P pools by the plant and the effect it has on soil biochemical characteristics. For instance, soil pH in the rhizosphere fundamentally regulates the adsorption-desorption process of Pi (Richardson et al. 2009), and soil microbes interact soil organic matter to mineralize Po (Jin et al. 2015). Therefore, whether chemical or biological processes dominantly contribute to the liberation of non-labile P may vary among soils differing in the composition of P fractions and organic matter content.

This study aimed to investigate the long-term effect of eCO_2 on soil P pools, and associated soil biochemical properties in a wheat-pulse rotation system in three major Australian soils subjected to FACE. We hypothesized that the decrease in P fractions under eCO_2 varied between soils because both the quantity of P removed as grains harvested and the changes in soil properties in response to eCO_2 would differ between the soils, favouring the mobilization of P from different P fractions to replenish labile P. In particular, Po in soils with high SOC contents (Chromosol) would be mineralized while non-labile Pi would be readily mobilized in soils with low SOC (Vertosol and Calcarosol) in which Po is not a major component of soil P.

Materials and methods

Experimental design

A free-air-CO_2-enrichment (SoilFACE) experiment was conducted from 2009 to 2015 in a medium-rainfall region, Horsham, Victoria Australia (36°44′57″S, 142°06′50″E). Four FACE bunkers were treated for elevated CO_2 (eCO_2) (550 ± 30 ppm) and the other four bunkers at ambient CO_2 (aCO_2) (390 ± 10 ppm), which represented four replicates. The eCO_2 level was automatically monitored and maintained from sunrise to sunset daily throughout the growth season. The FACE system used to achieve eCO_2 was detailed in Mollah et al. (2011).

Three major soil types within dryland cropping systems of South-Eastern Australia were included in this study, i.e. Chromosol, Vertosol and Calcarosol (Isbell 2002) or Luvisol, Vertisol and Calcic Xerosol (FAO-UNESCO 1976). The intact soil cores (0.3 m diameter; 1.0 m depth), which maintain the physicochemical integrity of the soil profile, were collected from paddocks, and secured in PVC sleeves. The intact soil cores were arranged into the bunkers sunk into the ground so that the top of each core was at the soil surface of the surrounding paddock. Each bunker contained up to 44 such cores, twelve of which were dedicated to this study (four for each soil). The soils were sampled after crop harvest in 2015 for the analysis of chemical properties, including total carbon (TOC), nitrogen (N), P fractions and pH. The Wimmera region where this experiment was located is characterised by a Mediterranean type climate with cool wet winters and

dry hot summers. Over the experimental period, the annual rainfall was in a range from 295 to 630 mm, maximum mean temperature from 21.4 to 23.1 °C, and minimum mean temperature from 7.2 to 8.1 °C (Fig. 1).

A wheat-pulse rotation was deployed in this study. Wheat (*Triticum aestivum* L. cv. Yitpi), field pea (*Pisum sativum* L. cv. PBA Twilight) and canola (*Brassica napus* L. cv. Hyola 50) were grown in the following rotation: field pea in 2009, wheat in 2010, field pea in 2011, wheat in 2012, canola in 2013, wheat in 2014, and canola in 2015. Phosphorus was applied as triple superphosphate at an annual rate of 15 kg P ha^{-1}. Nitrogen in urea was applied at 50 kg N ha^{-1} for wheat and canola but not for field pea. At maturity, plant shoots were cut-off at the base of the stems. After the removal of grains, the residues were chopped into pieces (less than 2 cm), and returned to their respective mesocosms in the field. Mesh netting was set to avoid the loss of residues during the summer period.

Grain harvest, soil sampling and measurements

Grains/seeds of wheat, filed pea and canola were harvested at maturity every year, dried, weighed and stored in plastic containers at 20 °C to avoid moisture absorption. Grain samples were dried at 70 °C for 72 h and then ground. Subsamples of ground grain were digested with a mixture of nitric and perchloric acid (4:1) (Yuen and Pollard 1954), and the concentrations of P in digests were analysed using an inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer Optima 8000, MA, USA). The concentrations of grain P were then used to calculate total P removal during the experimental period.

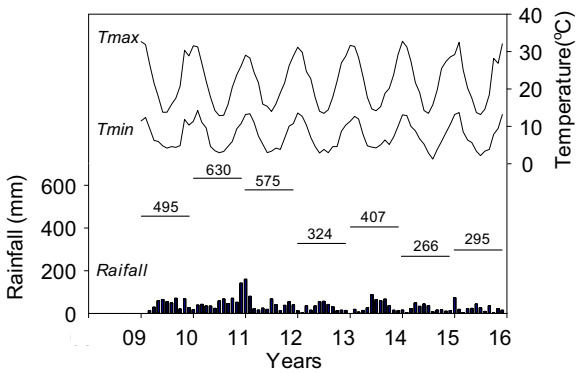


Fig. 1 Monthly and yearly rainfalls, and monthly minimal (*Tmin*) and maximal (*Tmax*) temperatures during the experimental period from 2009 to 2015. The data were obtained from the Bureau of Meteorology weather station 10 km from the experimental site

In December 2015 following crop harvest, surface soils (0–10 cm) were sampled. A composite sample for each replicate of individual soil types was obtained by combining soil cores from 4 mesocosms of each soil type in each bunker. Around 50 g soil of each sample was subsampled for a pre-incubation at field moisture prior to microbial measurements. Soils were watered to 60% field capacity, and incubated in plastic bags at 25 °C for 15 d. The bags were opened to allow gas exchange with ambient air. The remaining soil was air-dried, and passed through a 0.5-mm sieve for further chemical analyses.

After soil samples were incubated for 15 d, the microbial biomass carbon (MBC) was determined according to Vance et al. (1987). For microbial respiration measurement, 10 g of soil was put into a PVC core (4.5-cm height, 2-cm diameter) with nylon mesh bottom. The PVC core was placed into a sealed mason jar (237 ml) together with a vial containing 2 mL of CO_2-free water to maintain the humidity inside the jar. Soil was incubated at 25 °C for 24 h, and then the CO_2 concentration in the air-space of the jar was measured using an infra-red gas analyser (Servomex4210 Industrial Gas Analyser, Cowborough, UK) (Zibilske 1994; Rukshana et al. 2012). The microbial metabolic quotient was calculated as soil respiration rate divided by MBC.

Phosphorus fractionation was performed using air-dried soil using a modified Hedley scheme of P fractionation (Guppy et al. 2000). Inorganic P (Pi) within individual fractions were determined using malachite green (Motomizu et al. 1983). Total P in the bicarbonate ($NaHCO_3$) and hydroxide (NaOH) fractions were determined following digestion using acid ammonium persulphate in a microwave digester (100 kPa and 120 °C) for 45 min (Turner et al. 2003). The Po in these two fractions was calculated by subtracting the Pi from the total P. Total C (SOC) and N concentrations of soils were determined by dry combustion (Perkin Elmer 2400 Series II, USA) following grinding and homogenisation using a ball mill (Retsch MM400, Germany). Soil pH was determined using a pH meter (Thermo Orion 720A+, Beverly, MA, USA) after end-over-end shaking 1 g air-dried soil in 5 ml 0.01 M $CaCl_2$ for 17 h and centrifugation at 800 *g* for 5 min.

Statistical analyses

Data were analysed with Genstat 13 (VSN International, Hemel Hemspstead, UK). Two-way ANOVA (Steel and

Torrie 1980) was performed to determine the effects of CO_2, soil type and their interaction on grain P content, soil pH, SOC, total soil N, microbial properties and soil P fractions. Treatment means were compared using the least significant difference (LSD) at the significant level of $p = 0.05$.

Results

In general, the Chromosol had the highest total P whereas the Calcarosol had the lowest P content (Fig. 2). The largest P fraction was NaOH-Po for the Chromosol, residual-P for the Vertosol and $NaHCO_3$-Pi for the Calcarosol. Compared to the original status in 2009, seven years of P fertilizer application (by 2015) under aCO_2 significantly ($p = 0.05$) increased the concentrations of all P fractions except for residual-P. Under eCO_2, the concentration of soil P extractable with $NaHCO_3$ in the Chromosol were above but all other soil fractions were below the original 2009 level. In the other two soils, seven years of P fertilizer application generally increased the concentrations of all P fractions except for the residual P in three soils, and Po and total P in Calcarosol under eCO_2 (Fig. 2).

Compared to aCO_2, eCO_2 decreased $NaHCO_3$-extractable Pi in all three soils (Fig. 2a). The decrease ranged from 17 to 36% but there was no significant ($p > 0.05$) CO_2 × soil interaction observed (Table 1). The concentration of NaOH-Pi was significantly lower under eCO_2 than that under aCO_2 in the Chromosol and Vertosol soils but not in the Calcarosol (Fig. 2c; Table 1). After seven years of eCO_2 treatment, NaOH-Pi decreased from 81.8 to 63.1 mg kg^{-1} in the Chromosol, and from 22.2 to 5.1 mg kg^{-1} in the Vertosol. Although eCO_2 did not change $NaHCO_3$-extractable Po significantly in the soils (Fig. 2b), the response of NaOH-Po to eCO_2 varied between soils (Fig. 2d). As the largest soil P fraction in the Chromosol, the decreased amount of NaOH-extractable Po in this soil reached 20 mg kg^{-1} compared to only 12 and 7 mg kg^{-1} in the Vertosol and Calcarosol, respectively.

Elevated CO_2 decreased HCl-P and residual P with an average decrease of 4.5, 8.2 mg P kg^{-1} across the three soils, respectively (Fig. 2e, f). A similar trend was observed in total P in all three soils (Fig. 2g, Table 1).

As a total of 742 mg P was applied as fertiliser to each core over the 7 years, the total amounts of P removed by harvested grains under aCO_2 were 382, 462 and 246 mg P per core for the Chromosol, Vertosol, and Calcarosol, respectively (Fig. 3). However, eCO_2 resulted in an extra 134, 91 and 93 mg P $core^{-1}$ removal compared to aCO_2 for respective soils with a significant CO_2 × soil P interaction ($p < 0.05$) (Table 1).

The concentration of soil organic C (SOC) in the Chromosol decreased significantly ($p < 0.05$) over seven years, while there was no significant change in the other two soils. In 2015, the concentration of SOC in the Chromosol under aCO_2 was 42 g kg^{-1}, which was 4.8- and 12.1-fold higher than that in Vertosol and Calcarosol, respectively (Fig. 4a). Compared to aCO_2, eCO_2 decreased SOC concentration by 8.2% in the Chromosol, but did not affect it in the other two soils, leading to a significant CO_2 × soil interaction (Table 1). A similar trend was observed in total N content in soil (Fig. 4b). However, there was no significant CO_2 effect on C/N ratio in any soil (Fig. 4c). Interestingly, the CO_2 effect on C/P ratio was significant; eCO_2 increased the C/P ratio by 69% in Calcarosol but only by 10% and 12% in Chromosol and Vertosol, respectively (Fig. 4d).

Soil pH varied among the soils with 4.45, 7.32 and 5.75 (extractable in 0.01 M $CaCl_2$) for the Chromosol, Vertosol and Calcarosol soils, respectively. Soil pH did not change significantly over seven years under aCO_2, but increased by an average of 0.2 units under eCO_2 ($p < 0.05$) (Fig. 5; Table 1). The effect of eCO_2 on MBC depended on the soil type. The MBC in the Chromosol increased from 59 to 75 μg g^{-1} soil when crops were subjected to eCO_2, while it was not affected by CO_2 in the Vertosol or Calcarosol soil (Fig. 6a; Table 1). Soil microbial respiration in the Chromosol was 3.8- and 3.3-fold higher than in the Vertosol and Calcarosol soils, respectively (Fig. 6b). Elevated CO_2 increased soil respiration by 24% in the Chromosol and 53% in the Calcarosol, but did not affect it in the Vertosol. Elevated CO_2 increased the microbial quotient in the Calcarosol but not in the two other soils (Fig. 6c).

Discussion

The amount of P added to the soil-crop system as fertilizers exceeded the P removal in grains over the 7-year study period, contributing to a net accumulation of P in the top 10 cm of soil. The amount of accumulated P however was less than the estimated P remaining in the

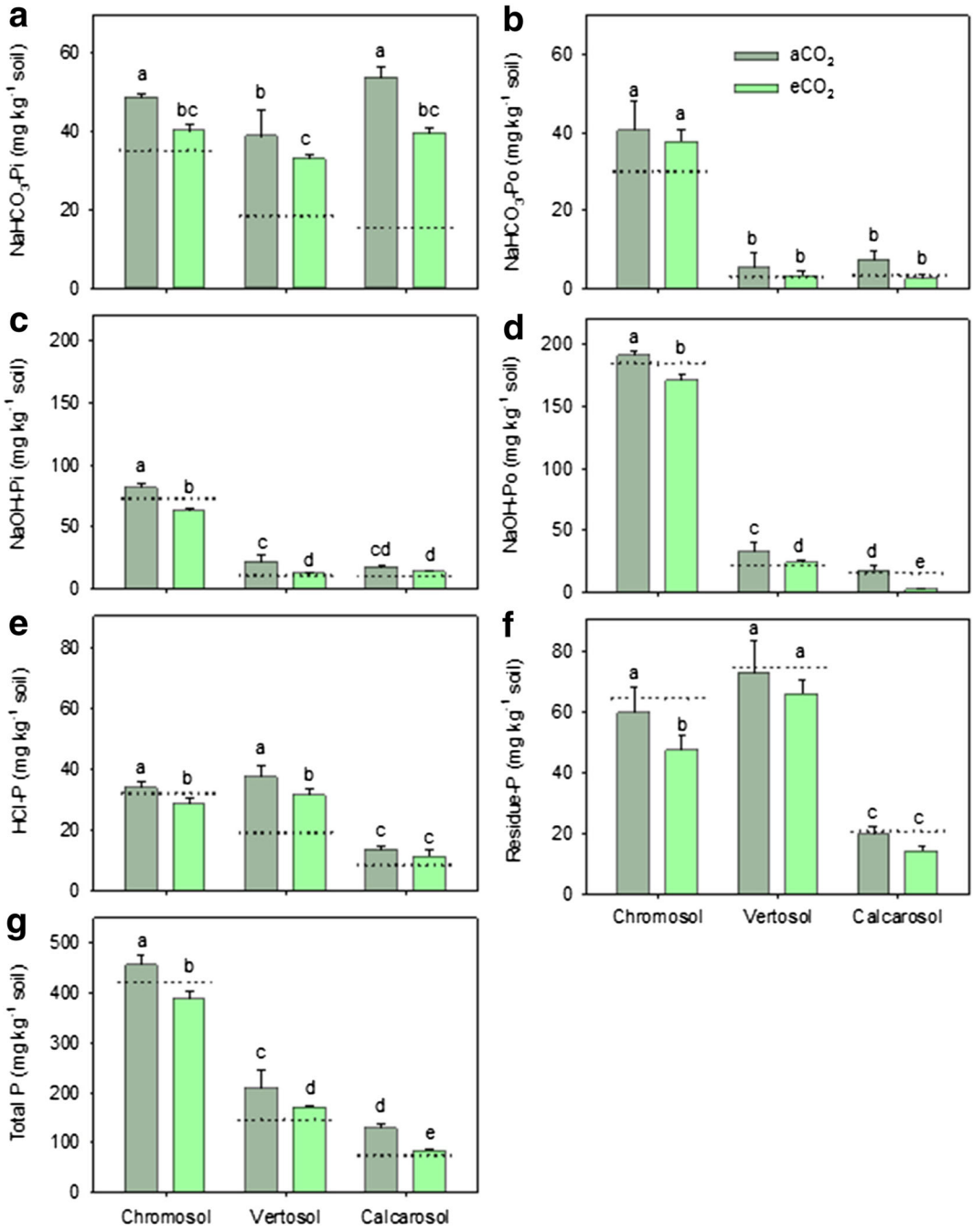


Fig. 2 The effect of 7-year eCO_2 on concentrations of $NaHCO_3$-Pi (**a**), NaOH-Pi (**b**), $NaHCO_3$-Po (**c**), NaOH-Po (**d**), HCl-Pi (**e**), residual P (**f**) and total P (**g**) in the Chromosol, Vertosol and Calcarosol soils. Crops were grown under FACE from 2009 to 2015. Dotted lines represent the initial value in each soil before the start of FACE in 2009. Values were means ± SE (n = 4). Values with a same letter are not significantly different between treatments (p = 0.05)

soil (Figs. 2 and 3). This was likely attributed to undecomposed plant residues, and possible P leaching through the soil profile (Sharma et al. 2017), especially in the Calcarosol due to low P-sorption capacity in sandy topsoil (Siemens et al. 2004; Liu et al. 2012).

When crops were grown under eCO_2 for 7 years, the total P in the topsoil decreased considerably compared

Table 1 Significant levels of main effects and interactions of CO_2 and soil type on P fractions, P removal, soil organic C (SOC), soil N, C/N, C/P, soil pH, and microbial properties

Variables	CO_2		Soil		CO_2 × Soil	
	M.S.	*p* values	M.S.	*p* values	M.S.	*p* values
$NaHCO_3$-Pi	542	< 0.01	256	< 0.01	39	0.40
NaOH-Pi	661	< 0.001	8321	< 0.001	123	< 0.05
$NaHCO_3$-Po	61	0.31	3150	< 0.001	3.6	0.94
NaOH-Po	808	< 0.001	71,718	< 0.001	218	< 0.01
HCl-P	119	< 0.05	1130	< 0.001	7.9	0.67
Residue-P	401	0.12	5766	< 0.001	26	0.84
Total P	15,096	< 0.01	221,013	< 0.001	830	0.51
P removal	27,111	< 0.001	87,772	< 0.001	492	< 0.05
SOC	4.6	< 0.05	2160	< 0.001	5.9	< 0.01
Soil N	0.03	< 0.001	14	< 0.001	0.03	< 0.001
C/N	1.1	0.32	29	< 0.001	2.6	0.11
C/P	446	< 0.001	6910	< 0.001	90	< 0.05
pH	0.14	< 0.05	13	< 0.001	0.01	0.49
MBC	304	0.07	15,572	< 0.001	325	< 0.05
Soil respiration	0.02	< 0.05	0.60	< 0.001	0.01	< 0.05
Metabolic quotient	2.9	0.97	141	< 0.001	4.8	< 0.05

M.S., Mean square; MBC, Microbail biomass C

to that at aCO_2 (Fig. 2). Greater P removal under eCO_2 contributed to this decrease of total P because larger quantities of grains were harvested under eCO_2 over time (Fig. 3). The amount of P removed from the Chromosol was the largest among the three soils in response to eCO_2, which was consistent with the largest decrease in total P in this soil compared to aCO_2 (Figs. 2 and 3). This suggests that crops primarily utilised soil P in this soil layer to produce grain gain under eCO_2. A

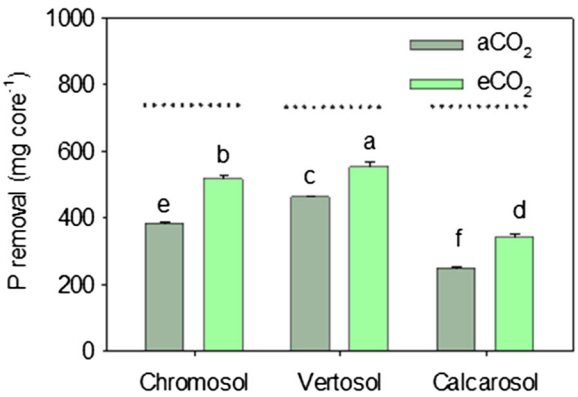
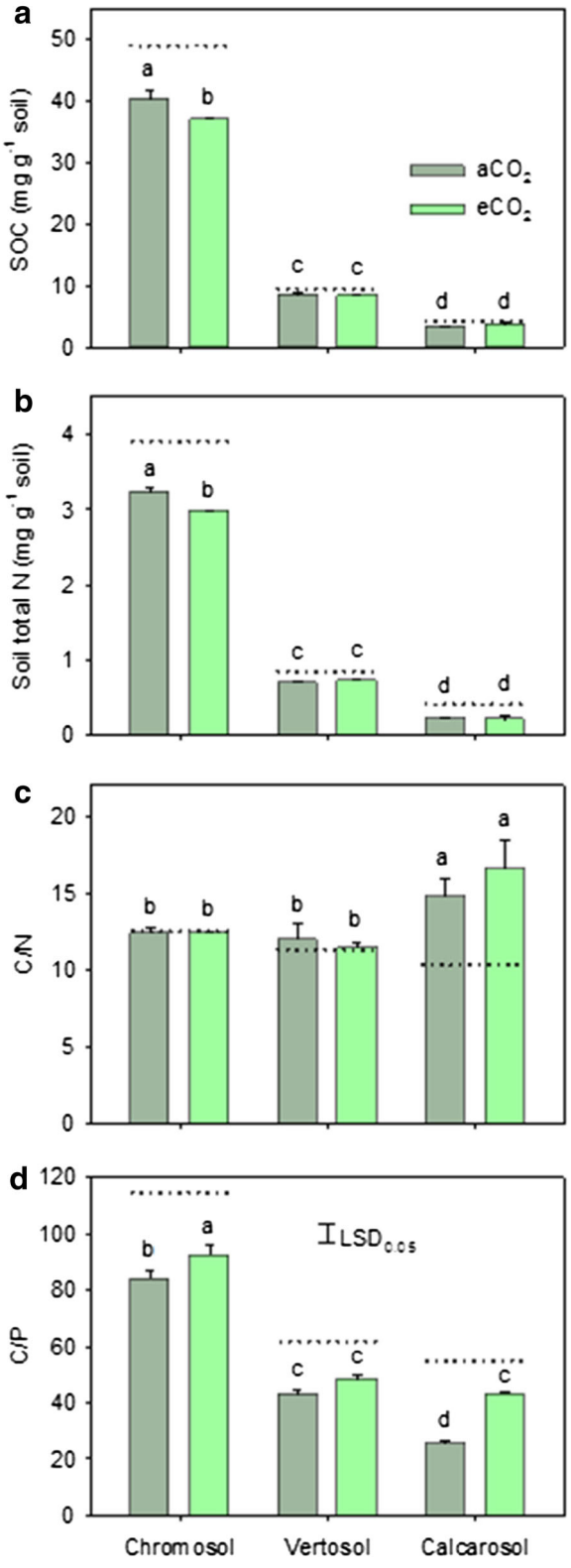


Fig. 3 The effect of 7-year eCO_2 on total P removal from the Chromosol, Vertosol and Calcarosol soils over the period from 2009 to 2015 due to the grain harvest. Values were means ± SE (n = 4). Values with a same letter are not significantly different between treatments (p = 0.05). Dotted lines represent the total P input as fertilizers in each soil during the experimental period

number of other studies have also shown that eCO_2 increased the growth and biomass of C3 plants and hence P removal when sufficient P was supplied (Jin et al. 2012; Jakobsen et al. 2016). In the present study, although total P decreased under eCO_2 compared to aCO_2, the total P in soils under eCO_2 did not drop significantly ($p > 0.05$) compared to the original soil in 2009. This indicates that the current P fertilizer rate was able to maintain the equilibrium of total P in this soil-plant system.

As the P contained in the grain harvested from the cores was the major source of P export, the majority of this P had to pass through the plant-available P pool, i.e. labile P. The eCO_2-induced decrease in P concentration occurred across all soil P fractions measured. Elevated CO_2 decreased the most-readily-available P fraction as well as sparingly-soluble P fractions (Fig. 2). This result was consistent with a previous study showing that eCO_2 decreased the recalcitrant P fractions to replenish the labile-P fraction in a forest ecosystem (Huang et al. 2014). Several studies have reported that the insoluble P associated with Fe and Al oxides and Ca compounds can be depleted by biotic P demand (Chen et al. 2000; Richter et al. 2006). For example, Vu et al. (2008) found that wheat and chickpea grown in a Calcarosol over two growth cycles depleted P in all soil P fractions that were assessed. Thus, it can be deduced from this present



◀ **Fig. 4** The effect of 7-year eCO_2 on soil organic C (SOC), total soil N, C/N ratio and C/P ratio in Chromosol, Vertosol and Calcarosol. Crops were grown under FACE from 2009 to 2015. Values were means ± SE ($n = 4$). Values with a same letter are not significantly different between treatments ($p = 0.05$). Dotted lines represent the initial value of each soil before the start of FACE in 2009

study that a long-term exposure to eCO_2 facilitated P uptake by plants from the labile P fractions which in turn accelerated the desorption and/or dissolution of P from the non-labile fractions through biochemical equilibria in soils.

The magnitude of the eCO_2-induced decrease in P fractions, however, depended on soil type. Elevated CO_2 decreased the size of the NaOH-extractable Pi pool in the Chromosol and Vertosol (Fig. 2). The NaOH-Pi is considered as a main fraction representing residual fertilizer P in soils, and P in this fraction is primarily absorbed by sesquioxides (Parfitt 1978; Khan et al. 2008; Zhang et al. 2006), such as Al_2O_3 and Fe_2O_3 in soils (Perrott et al. 1989; Vu et al. 2009). When the depletion of labile P was accelerated by plants grown under eCO_2, NaOH-Pi might be reversibly desorbed into the labile Pi pool to meet increased P demands by crop plants. This view is also supported by previous studies that intensive cropping depleted NaOH-Pi and $NaHCO_3$-Pi in a number of soils including Vertisol, Mollisols, Ultisols and Oxisols (Guo et al. 2000; Vu et al. 2008). Moreover, the small increase in soil pH under eCO_2 (Fig. 5) in this current study might help to

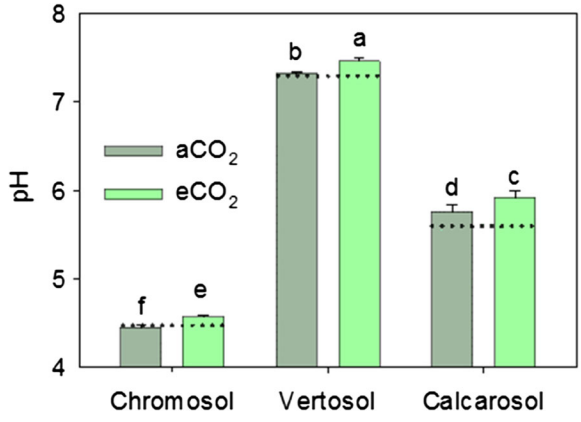


Fig. 5 The effect of 7-year eCO_2 on the pH of Chromosol, Vertosol and Calcarosol. Crops were grown under FACE from 2009 to 2015. Values were means ± SE ($n = 4$). Values with a same letter are not significantly different between treatments ($p = 0.05$). Dotted lines represent the initial value of each soil before start of FACE in 2009

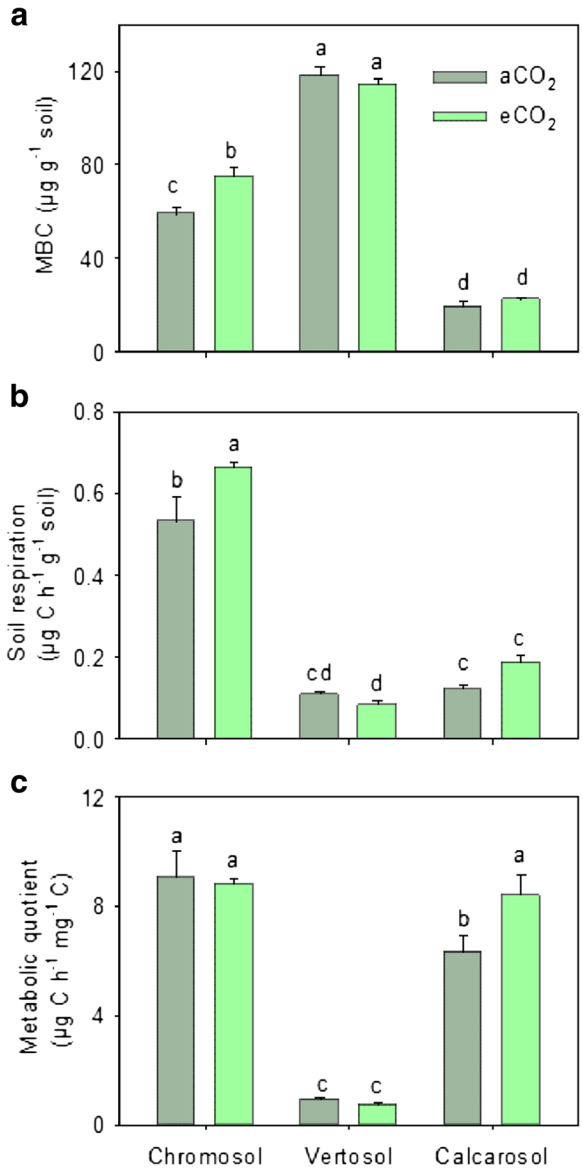


Fig. 6 The effect of 7-year eCO_2 on microbial biomass C (MBC) (**a**), soil basal respiration (**b**), and metabolic quotient (**c**) in Chromosol, Vertosol and Calcarosol. Crops were grown under FACE from 2009 to 2015. Values were means ± SE ($n = 4$). Values with a same letter are not significantly different between treatments ($p = 0.05$)

facilitate the desorption of P from the NaOH-Pi fraction in acid soils, rather than P sorption or precipitation (Gentile et al. 2012). This appeared to occur in the Chromosol where the pH was less than 4.5. However, the size of the NaOH-Pi pool in the Calcarosol did not change greatly in response to eCO_2. This may be attributed to the small size of NaOH-Pi in this particular soil (Fig. 2), limiting the sorption and dissolution of P in this fraction. Furthermore, the major P fraction in the Calcarosol was $NaHCO_3$-Pi, which accounted for more than 40% of total P. The decrease of labile P did not reach the point triggering P transformation from NaOH-Pi because the effectiveness of Al-P/Fe-P in the NaOH-Pi fraction as a P source might depend on the concentration of $NaHCO_3$-Pi which affected the equilibrium of P between the fractions.

Over 7 years of CO_2 enrichment, the size of the NaOH-Po pool declined in all three soils, especially in the Chromosol in term of the amount of Po decline. This indicates that mineralization of Po became one of major sources of P replenishment of available P, as the NaOH-Po was the primary P fraction in the Chromosol, representing 41% of total P. The Chromosol was more prone to SOC decomposition (Fig. 4), which might be triggered by increased labile C inouts under eCO_2 (Jin et al. 2015; Vestergard et al. 2016). Since eCO_2 increased plant biomass production in the Chromosol, which had greater SOC concentration than other two soils (Fig. 4), the resultant larger amount of C efflux from the crop roots in this particular soil might induce a stronger priming effect (van Groenigen et al. 2014; Vestergard et al. 2016). The eCO_2-induced increases in microbial biomass C and microbial activity in the Chromosol (Fig. 6) appears to be associated with a priming effect. Moreover, eCO_2 induced changes in bacterial community composition in plant rhizosphere (Yu et al. 2016), while the change in bacterial community structure and functions (Nie et al. 2013; Pendall et al. 2013) are likely to be associated with decomposition of organic matter (Lian et al. 2017). The biological/biochemical processes during SOC decomposition would lead to P mineralisation (Bünemann 2015). This speculation was supported by the decrease of SOC and NaOH-Po in this soil under eCO_2 (Fig. 4), and the fact that eCO_2 facilitated the transformation from Po to Pi in an Inceptisol (Khan et al. 2008).

Although the NaOH-Po fraction in the Calcarosol was not the major P fraction, this fraction experienced a decrease in size in response to eCO_2 as well (Fig. 2). The mechanism of how microbial activity contributed to the P depletion is most likely different from that of the Chromosol, since the microbial quotient in this soil was greater than that of the Chromosol under eCO_2 compared to aCO_2 (Fig. 6). This indicates that specific microbial groups might be responsible for mineralizing Po. However, the hypothesis is yet to be tested. In the Vertosol, a decrease of NaOH-Po was observed as well, but the biochemical mechanisms are inconclusive due to

the insignificant response of microbial properties and SOC to eCO_2 (Figs. 4, 6). As the response of this fraction to eCO_2 was marginal compared to other fractions such as NaOH-Pi and HCl-P, the impact of eCO_2 on P transformation in this soil appears to be mainly associated with physiochemical processes occurring between the P fractions, rather than biological effects.

Our previous study (Jin et al. 2013) showed that eCO_2 increased NaOH-Po in the rhizosphere of wheat grown in a Vertosol. The discrepancy between this present and previous studies may be attributed to the status of P in response to eCO_2. During the plant growth under eCO_2, the microbes temporarily immobilized P in the rhizosphere due to an increased labile C efflux from roots. The major component of increased Po was likely microbial P associated with diesters such as DNA and/or RNA. Soil microbial biomass has been reported as a labile Po that can contribute to plant available P (Macklon et al. 1997; Kritzler and Johnson 2010). The microbial P would then be re-mobilised over time with the biomass turnover. Strong evidence from both arid ecosystems (Attiwill and Adams 1993) and laboratory microcosms in a grass species (Macklon et al. 1997) suggests that the mineralization of these labile Po can supply large amounts of available P to plants. In the long term, net P mineralization may occur following decomposition of the indigenous SOC in soils, such as the Chromosol under eCO_2. Wang et al. (2016) found the significant correlation between Po and C concentrations in Regosols, indicating that mineralisation of Po and organic C were linked each other, and P may be released as the by-product of C mineralization. Thus, the Po compounds bond to soil organic matter such as inositol phosphates may be gradually mineralized due to stimulated phosphomonoesterases with plant succession under eCO_2. However, greater C/P ratios under eCO_2 suggest that eCO_2-induced mineralisation of SOC is stronger than their mining Po sources. How the eCO_2-induced priming effect on SOC is associated with P mineralization and how microbial functions contribute to the C/P association warrant further investigation.

Although soil residual P contains occluded inorganic and stable organic forms of P (Chen et al. 2000) and is unlikely to contribute to soil solution P and plant P nutrition in the short-term (McDowell and Condron 2000), long-term exposure to eCO_2 tend to decrease this fraction in all three soils. This is consistent with some of the results from Khan et al. (2008) and Huang et al. (2014). The results indicate that greater biotic P demand under eCO_2 leads to a decrease of residual P to sustain soil labile P fractions. Plant growth and P uptake were strongly associated with most P fractions, including the residual P (Vu et al. 2008). A number of studies presented evidence that the depletions in the residual P occurred after exhaustive P uptake (Mckenzie et al. 1992; Zhang et al. 2006). Thus, long-term cropping under eCO_2 likely facilities crop access to recalcitrant P forms.

Conclusions

Compared to aCO_2, 7-year exposure of eCO_2 in cropping systems led to a decrease of soil P due to greater crop removal in three major soils used for grain production in south-eastern Australia. The size and nature of the decrease of P in different fractions varied between the soils. In the Chromosol, the NaOH-Pi and NaOH-Po fractions were the dominant fractions which were decreased by eCO_2, while decreases in $NaHCO_3$-Pi and NaOH-Pi occurred in the Vertosol. In the Calcarosol, the major decrease of P was recorded in the $NaHCO_3$-extractable Pi fraction. These results indicate that biochemical processes were involved in the P transformation in the Chromosol while physiochemical processes dominated in the Vertosol and Calcarosol. Due to the significant changes in SOC and Po in the Chromosol, the proper strategies of P management such as the use of Po-enriched organic amendments should be particularly concerned for this type of soils to sustain grain production in future eCO_2 environments.

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