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Increased microbial activity contributes to phosphorus immobilization in the rhizosphere of wheat under elevated CO_2

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Abstract
Understanding phosphorus (P) transformation in the rhizosphere affected by elevated CO_2 (eCO_2) needs to underpin the plant-derived C flow and P relationship in the plant-soil-microbe continuum. A pot experiment was conducted in CO_2-controlled environmental cabinets. Wheat (*Triticum aestivum* L.) plants were grown in a P-sufficient Vertisol soil and exposed to 380 or 800 ppm CO_2 for 6 weeks. Plants were labelled with $^{13}CO_2$ under respective CO_2 treatments. Elevated CO_2 increased $NaHCO_3$- and NaOH-extractable organic P (Po) in the rhizosphere by 160% and 53%, respectively. Consistently, eCO_2 increased microbial C and respiration in the rhizosphere. Furthermore, the excess of ^{13}C atom in roots and rhizosphere soil, but not in shoots, were markedly higher under eCO_2 than aCO_2. Elevated CO_2 increased the copy number of bacterial 16S rDNA from ^{13}C-DNA and ^{12}C-DNA fractions. Although the copy number of fungal 18S rDNA from ^{13}C-DNA was higher under eCO_2, there was no difference on the copy number of total 18S rDNA between the CO_2 treatments. It is concluded that the increased Po in the rhizosphere under eCO_2 was mainly attributed to stimulating microbial biomass/activity which in turn immobilized more P and root-derived materials. The stimulation of microbes resulted from increased C efflux from root systems under eCO_2.

Key words: ^{13}C labelling, carbon allocation, climate change, CO_2 concentration, microbial activity, P transformation, root exudates

1. Introduction

Understanding the impact of elevated CO_2 (eCO_2) on nutrient cycling in soil can help identify strategies needed to maintain soil nutrient supply in future cropping systems. For example, nitrogen (N) cycling is affected by eCO_2; N immobilization can occur in soils when plants grown under eCO_2 and this immobilization may limit plant growth in response to eCO_2 in the long term (De Graaff et al., 2006). However, research on how eCO_2 influences P acquisition of crops in soils is scarce. Recently, Jin et al. (2012; 2013) showed that eCO_2 enhanced P demand of crop plants and markedly increased extractable organic P (Po), an indication of P immobilization, in the rhizosphere. However, the mechanisms leading to the increase of Po are unclear.

Understanding how Po is increased under eCO_2 will assist in optimizing P management in farming systems in future climate changed environments because Po comprises a large part of total soil P, and can be transformed into labile Pi (Ahlgren et al., 2013; Achat et al., 2010). There are three possible causes of the increase in Po in the soil. Firstly, one source of the increased Po may be rhizodepositions. Johansson et al. (2009) reported that eCO_2 increased root exudates of *P. sylvestris* (L.) Karst with low-molecular-weight organic compounds increasing by 120−160%, dissolved organic carbon by 180−220% and amino acids by 250%. These root-derived substances could be P-enriched. Secondly, decomposition of soil organic matter could also contribute to the increased Po under eCO_2. Elevated CO_2 may induce a priming effect via the efflux of labile substrates from roots to the rhizosphere, accelerating the decomposition of soil organic matter, but this process is likely to be greatly limited by the low organic C content in the tested soil (Jin et al., 2012). Thirdly, increased microorganism population in the rhizosphere could be an important cause of increased Po under eCO_2. A study using *Lolium perenne* showed a 42% increase in microbial biomass in soil after plants were exposed to 700 ppm CO_2 for 3 months (van Ginkel et al., 2000). It was evident that the microbial C significantly increased under eCO_2 and correlated positively with an increase in Po fractions (Jin et al., 2013). Srivastava and Singh (1988) also reported that

microbial biomass accounted for 9.2–19.2% of the total Po in six tropical soils and that microbial P was positively related with $NaHCO_3$-Po. However, how eCO_2 regulates the microbes to immobilize P or alters P-use efficiency by microbes remains unknown. This would be associated with the contribution of rhizospheric C flow to the accumulation of microbial P, and major microbial structure contributing to such Po accumulation under eCO_2.

The $^{13}CO_2$ labelling technique quantifies the fate of plant-assimilated C in plant-soil-microbe continuum (Subedi et al., 2006). Further, this technique can also be used to trace the incorporation of plant-originated C fluxes into microbial nucleic acids (^{13}C stable isotope probing, SIP), by which the quantity and nature of microbes utilizing root-derived substances can be identified (Boschker et al., 1998; Vandenkoornhuyse et al., 2007; Whiteley et al., 2007). By using the $^{13}CO_2$ labelling in this study, the effect of eCO_2 on C flow in soil microorganisms and its contribution to P transformation in the rhizosphere can be assessed. We hypothesize that the eCO_2-induced increase of the photosynthetic C efflux from root system stimulates microbial biomass/activity. Thus, the demand for P by microbes may subsequently increase.

2. Materials and Methods

2.1. Experimental design and plant growth

A pot experiment was undertaken in growth chambers. The experiment design consisted of two CO_2 levels, i.e. ambient CO_2 (aCO_2; 380 ppm) and elevated CO_2 (eCO_2; 800 ppm) in a randomised design. Each treatment consisted of eight replications with plants and four without plants as bulk soil controls. The plant species was wheat (*Triticum aestivum*. L. cv. Beaufort).

A Vertisol (FAO – UNESCO, 1976) soil was collected from a field site that had been part of a long-term phosphorus fertilisation trial in Horsham, Victoria (36°42'S, 142°11'E). The soil organic C was 13.3 g kg^{-1}, total N 1.1 g kg^{-1}, total P 254 mg kg^{-1}, Olsen P 43 mg kg^{-1} and a pH (1:5 in 0.01 M $CaCl_2$) 6.9. The soil was initially air-dried and then sieved (≤ 4 mm), before mixing with siliceous sand (w:w=1:1) to aid the collection rhizosphere soil at harvest. Each pot contained 4.5 kg of the soil/sand mix. Basal nutrients were added to all the pots in the following composition (mg kg^{-1}): urea, 60; K_2SO_4, 147; $CaCl_2$, 186; $MgSO_4.7H_2O$, 122; $MnSO_4.H_2O$, 6; $ZnSO_4.7H_2O$, 8; $CuSO_4.5H_2O$, 6; $CoCl_2.6H_2O$, 0.4; $FeCl_3$, 0.6; $NaB_40_7.10H_2O$, 1.6; and $Na_2MoO_4.2H_2O$, 0.4.

Wheat seeds were germinated for 1 d on moistened filter paper. Twenty geminated seeds were sown in each pot and thinned to 15 plants 7 days after emergence. All pots were allocated into 4 controlled-environmental cabinets (Fitotron SGC120, Weiss Gallenkamp Ltd, Leicestershire, United Kingdom) with 2 cabinets set at aCO_2 and 2 at eCO_2. Plants were grown at 22 °C (day, 12 h) and 18 °C (night) for 6 weeks with a light intensity (over the waveband 400–700 nm) of 550 μmol photons m^{-2} s^{-1}. Soil water content was maintained daily at 80±5% of field capacity (26% w/w) by weighing while the field capacity was determined according to Klute (1986).

2.2. $^{13}CO_2$ labelling

The plants were labelled with $^{13}CO_2$ for 5 days before harvest. Air-tight clear plastic chambers (area 50 × 54 cm^2, height 68 cm) were used for the labelling. On the labelling dates, half of pots with or without plants were moved into chambers and labelled with $^{13}CO_2$

which was generated by injecting 9.2 M H_2SO_4 into calculated amount of $Na_2{}^{13}CO_3$ with a syringe through a rubber gasket into the chamber (Subedi et al., 2006). A 67% of $^{13}CO_2$ abundance in chamber was remained in both CO_2 treatments, and the final concentrations of total CO_2 were 600 and 900 ppm for aCO_2 and eCO_2, respectively. A fan was installed inside each chamber to facilitate mixing of the ^{13}C label. Both planted and no plant controls were exposed to $^{13}CO_2$ for 3 hours each labelling event with two events per day. The other half of the pots were not subject to ^{13}C labelling (subsequently referred to unlabelled controls).

2.3. Harvest and measurements

After 6 weeks of growth post sowing (including 5 days of ^{13}C labelling), shoots of the wheat plants were cut at ground level and processed as described in Tang et al. (1990). Roots in each pot were carefully removed and the rhizosphere soil was recovered by gently shaking the roots. Each soil sample was separated into three parts: one part (around 50 g) was kept in 4 °C for microbial biomass and respiration measurements; one (around 15 g) was air-dried for P fractionation and ^{13}C measurements, and the third sample (around 3 g) was frozen immediately following harvest in liquid nitrogen, and stored at -80 ºC for the analysis of microbial molecular properties.

Roots were washed with deionized water to remove any adhered soil. Root morphology was measured following scanning with an EPSON EU-35 scanner (Seiko Epson Corp., Japan), and images analysed using 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 prior to determining P concentration colorimetrically (Motomizu et al., 1983) following acid digestion (Yuen and Pollard, 1954) .

Air-dried soil samples were mixed thoroughly and finely ground before further analysis. Soil P fractionation for samples was performed using a modified Hedley sequential P fractionation scheme (Guppy et al., 2000; Butterly et al., 2009). The stable ^{13}C isotope ratios in soil and plant samples were analyzed with an isotope ratio mass spectrometer (Delta^{plus}, Finnigan MAT GmbH, Bremen, Germany).

With fresh soil samples, the microbial biomass carbon (MBC) was extracted by chloroform fumigation 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 is calculated as the difference in TOC concentration in extracts between fumigated and non-fumigated soils. Microbial P was measured according to Brookes et al. (1984).

For microbial respiration measurement, 10 grams of fresh soil sample and 2 ml of CO_2-free water were added separately into two plastic vials. The two vials were placed into a sealed half-pint (237 ml) mason jar. Soil was incubated for 24 h at 25°C, and then the CO_2 concentration in the jar was measured using an Infra-red Gas Analyser (Servomex 4210 Industrial Gas Analyser, Cowborough, UK) (Zibilske, 1994; Rukshana et al., 2012).

Total DNA was extracted from frozen soil samples using the FastDNA SPIN kit for soil (MP BIomedicals; Solon, OH, USA) following the manufacturer's instructions. Total DNA was quantified using a NanoDrop Sqectrophotometer (Bio-Rad Laboratories Inc.). Five micrograms of DNA of each soil sample was loaded into 4.9 ml of cesium trifluoracetate (CsTFA) (Sigma) solution with a starting buoyant density of 1.60 g ml^{-1}. Density gradient centrifugation was performed in a Vti-65.2 vertical rotor at 179,000 *g* (43,500 rpm) for 40 h

at 20°C (Sul et al., 2009). After centrifugation, the gradients were fractionated according to Dunford and Neufeld (2010). Then, the DNA from heavy and light fractions were quantified for bacterial 16S rDNA (ribosomal DNA) and fungal 18S rDNA by quantitative PCR using the Absolute QPCR SYBR green mix (Ab Gene, Epsom, UK) (Sul et al., 2009). Regarding the standard of quantitative PCR, extracted rhizosphere DNA was amplified by PCR. The PCR product was cloned into *Escherichia coli*. One *Escherichia coli* clone containing an insert of bacterial or fugal origin was randomly chosen and went through a colony PCR procedure using the plasmid enclosed primer. This PCR product was then used to make a series of known concentrations of a standard (Drigo et al., 2007). The primers of PCR were 357f and 517r for bacterial 16S rDNA (Muyzer et al., 1993) and ITS1-F and ITS2 for fungal 18S rDNA (Gardes and Bruns, 1993).

2.4. Statistical Analysis

Statistical analysis was performed with Genstat 13 (VSN International, Hemel Hemspstead, UK). A two tailed t-test was used to assess the differences between the two CO_2 treatments (one-way ANOVA) (Steel and Torrie, 1980).

3. Results

3.1. Plant growth and P uptake

Elevated CO_2 increased shoot dry weight by 47% (Fig. 1a), and root dry weight by 188% (Fig. 1b). Consequently, the root-to-shoot ratio increased from 0.39 under aCO_2 to 0.77 under eCO_2 (Fig. 1c). Root length increased by 58% under eCO_2 compared to aCO_2 (Fig. 1d).

Plant P concentration and P uptake were strongly affected by eCO_2. Compared to aCO_2, eCO_2 decreased P concentrations in shoot by 21% and in roots by 20% (Fig. 2a, b). Plant P concentrations under both CO_2 treatments were above the critical level of P deficiency for this stage of growth (Reuter and Robinson, 1997). Elevated CO_2 increased total P uptake by 35% due to the increased biomass production (Fig. 2c). However, eCO_2 did not significantly affect P uptake per unit root length (Fig. 2d), which averaged 0.11 mg P m^{-1}.

3.2. P fractionation in the rhizosphere

Although $NaHCO_3$-Pi was lower in the rhizosphere than the unplanted soil, elevated CO_2 did not affect other P fractions (Fig. 3a). However, organic P (Po) extracted by $NaHCO_3$ ($NaHCO_3$-Po) and NaOH (NaOH-Po) accumulated in the rhizosphere when plants were grown under eCO_2 (Fig. 3). Compared to aCO_2, eCO_2 increased $NaHCO_3$-Po by 160% (Fig. 3b) and NaOH-Po by 53% (Fig. 3d). Compared with the no-plant control, NaOH-Po increased in the rhizosphere of wheat under both aCO_2 and eCO2 while $NaHCO_3$-Po increased only under eCO_2.

3.3. Microbial properties

Microbial P in the rhizosphere increased from 5.2 mg kg^{-1} under aCO_2 to 7.1 mg kg^{-1} under eCO_2, a 37% increase (Fig. 4a). Microbial C exhibited the same pattern with a 29% increase under eCO_2 compared to aCO_2 (Fig. 4b). Hence, microbial C/P ratio in the rhizosphere was not altered by eCO_2 (Fig. 4c). In addition, microbial P was higher while microbial C/P ratio was lower in the rhizosphere compared to the no-plant control.

Elevated CO_2 markedly increased microbial respiration in the rhizosphere with 0.6 µg C h^{-1} g^{-1} soil, double that measured under aCO_2 (Fig. 5a). Similarly, the metabolic quotient of microbes in the rhizosphere increased to 2.8 mg g^{-1}, i.e. a 65% increase, when plants were

grown under eCO_2 (Fig. 5b). Metabolic quotient was higher in the rhizosphere than the unplanted soil, whereas the rhizosphere effect on microbial respiration was significant only under eCO_2.

3.4. ^{13}C allocation

Elevated CO_2 increased the allocation of ^{13}C into roots and rhizodeposition. Although ^{13}C atom‰ excess in shoot was not affected by CO_2 treatment (Fig. 6a), eCO_2 increased ^{13}C atom‰ excess in roots by 57% (Fig. 6b). The greater allocation of ^{13}C to roots under eCO_2 also resulted in 0.28 atom‰ excess in the rhizosphere, double that under aCO_2 (Fig. 6c). The ^{13}C atom‰ excess in the control soil without plants did not significantly differ between aCO_2 and eCO_2, and was significantly ($P < 0.001$) lower compared to soil where plants were present, resulting in only 0.02 atom‰ excess on average. The amount of ^{13}C allocated to root or efflux into soil was 111% higher under eCO_2 than aCO_2 (data not shown).

It was evident that the fixed ^{13}C was incorporated into soil microorganisms. Elevated CO_2 increased the copy number of 16S rDNA from ^{13}C-DNA and ^{12}C-DNA fractions by 76% and 78%, respectively (Fig. 7a). Elevated CO_2 also increased the copy number of 18S rDNA that was determined from ^{13}C-DNA fractions, while there was no difference on the copy number of total 18S rDNA between aCO_2 and eCO_2 (Fig. 7b).

4. Discussion

This study found that Po associated with readily labile P fractions ($NaHCO_3$- and NaOH-extractable) increased in the rhizosphere of wheat grown in a P-sufficient Vertisol clay soil under eCO_2. The results are consistent with our previous study which found an increase in the NaOH-Po pool in the rhizosphere of two legumes − chickpea and field pea grown under eCO_2, when P was supplied to a P-deficient soil (Jin et al., 2012). This suggests that some P in the rhizosphere is temporally immobilised under eCO_2.

It has been speculated that the immobilization of P in the rhizosphere under eCO_2 would be largely attributed to rhizosphere-associated microorganisms. This study confirmed this assumption and showed that microbial P increased proportionally with microbial biomass in response to eCO_2 (Fig. 3). No difference on microbial C/P ratio between aCO_2 and eCO_2 was observed (Fig. 3), further indicating that the increase in microbial P in the rhizosphere soil was due to the increasing microbial biomass rather than enhanced efficiency of P accumulation by microbes. The pattern of P utilised by microbes was not influenced by eCO_2. Thus, eCO_2 stimulates microbial populations, which in turn temporarily immobilize P.

The turnover of microbial P is largely driven by the availability of C (Richardson and Simpson, 2011). The turnover time of microbial biomass in cropping soils varied from 42 to 160 d due to C amendment (Oehl et al., 2004). Drigo et al. (2008) stated that this stimulation was because soil microorganisms are often C-limited. Increased C availability in the rhizosphere under eCO_2 occurred in this study as ^{13}C abundance in the rhizosphere of wheat almost doubled under eCO_2, and there was no accumulation of ^{13}C in the soil in the no-plant controls (Fig. 6). This affirmatively infers that roots exude more C compounds under eCO_2, which significantly stimulates metabolic processes of microbes, and may consequently alter the turnover of microbial P.

Microbial ^{13}C-DNA was separated from total DNA in this present study to investigate the

relative contribution of the plant root exudates to direct stimulation of microbial community in the rhizosphere. The copy numbers of 16S-rDNA (bacterial) and 18S-rDNA (fungal) in the ^{13}C fractions were significantly higher under eCO_2 than aCO_2 (Fig. 7), but the proportion of ^{13}C incorporation into bacteria was higher than for fungi (Fig. 7). The result indicates that the influence of eCO_2 on microbial community in the rhizosphere was predominantly on bacteria, rather than fungi during the short-period exposure of plants to eCO_2 in this study. The increase in root exudates under eCO_2 stimulated C flow between the plant and the bacterial population in the rhizosphere. The increase in microbial respiration of the rhizosphere soil from the eCO_2 treatment also supports that the increased C efflux as root exudates and rhizodeposits under eCO_2 stimulated microbial activity. In control soil without plants under eCO_2, only negligible amounts of ^{13}C were detected in the soil (Fig. 6). Quantitative alterations in C supply have been shown to increase microbial biomass and activity (Zak et al., 1993; Hungate et al., 2000; van Ginkel et al., 2000), and nutrient cycling (Jones et al., 1998).

Interestingly, eCO_2 appears to alter the structure of microbial communities. For example, the abundance of the active *Pseudomonas* in the rhizosphere increased under eCO_2, including *P. fluorescens*, *P. aeruginosa*, *P. trivialis* and *P. tutida* (Drigo et al., 2009). Treseder (2004) also reported that mycorrhizal fungal abundance increased relative to root length in response to eCO_2. These microbial genus were considered to be important in P transformations (Egamberdiyeva and Höflich, 2003; Krey et al., 2013). However, the identity of the specific species of microorganisms in the rhizosphere underpinning P immobilization in response to eCO_2 requires elucidation.

In this study, the extent of microbial P increase in response to eCO_2 was less than that of $NaHCO_3$-Po and NaOH-Po (Figs. 3 and 4). This may be partially attributed to the incomplete extraction of microbial P with the fumigation method, but the total microbial P estimated by a factor of k_{EC} (Joergensen, 1996) was still lower than Po. Thus, changes in microbial P cannot fully explain the increased Po in the rhizosphere. This could be attributed to two causes. Firstly, cell debris following microbial death can be a cause of the increases in Po (Beck and Sanchez, 1994; Boschetti et al., 2009). In this study, the microbial respiration rate and metabolic quotient were greater under eCO_2 than aCO_2 (Fig. 5), indicating that the turnover of microbes in the rhizosphere was increased by eCO_2. This increased turnover of microbes could generate more microbial residues and this is likely to further facilitate the accumulation of various Po forms including mononucleotides, nucleic acids and phospholiqids, which are microbial origin, and extractable by $NaHCO_3$ and NaOH (Turner et al., 2003; Romanyà and Rovira, 2009).

Secondly, root-derived P could also contribute to the increased Po under eCO_2 (Jin et al., 2013). Boschetti et al. (2009) assessed changes in soil P fractions by *Lotus corniculatus* and found that the Po concentration was positively and significantly correlated with root biomass, indicating that the increase in Po in the rhizosphere was related to root production. In this present study, higher root-to-shoot ratio under eCO_2 (Fig. 1) indicates the enhanced allocation of C belowground (Fig. 6). The increased ^{13}C enrichment in the rhizosphere of wheat in response to eCO_2 further confirmed that root-derived materials increased markedly (Fig. 6). These organic compounds include sloughing of living cells and exudation (Lynch and Whipps, 1990), which are likely to be P-enriched because of the relative high P concentration of P found in plant tissues compared to soil. Although plant P concentration was higher under aCO_2 than eCO_2 (Fig. 2), the rate of accumulation of root-derived materials in the rhizosphere was higher under eCO_2, because eCO_2 level significantly increased ^{13}C efflux per unit root length (data not shown). Thus, total amount of P-enriched materials from roots accumulated

in the rhizosphere under eCO_2. However, to date, the quantity and composition of P-enriched compounds in response to eCO_2 have not been elucidated. The use of ^{31}P NMR (nuclear magnetic resonance spectroscopy) may help identifying specific organic P compounds and their origins in soil samples (Vestergren et al., 2012).

Elevated CO_2 did not affect the $NaHCO_3$-Pi depletion in the rhizosphere, which coincided with nil CO_2 effect on P uptake per unit root length (Figs. 2, 3). This indicates that the additional P uptake by plants grown under eCO_2 (Fig. 1) came from greater soil exploration by roots. Greater root biomass and length under eCO_2 further support this view (Fig. 1). Similar results were also found in a previous study on chickpea and field pea (Jin et al., 2012). It is worthy to note that these results were obtained under P sufficient conditions. When plants were supplied with deficient P, there was no additional P uptake observed under eCO_2. This was probably because P deficiency diminished plant growth response to eCO_2 and P concentration in plant tissues did not differ between aCO_2 and eCO_2 (Jin et al., 2013).

However, the increased Po in the rhizosphere under eCO_2 may specifically contribute to the additional P uptake, since it may be potentially available to plants. With enhanced metabolic activity of microbes under eCO_2 (Fig. 5), a large proportion of this P-enriched materials could be mineralized over time. This is because most plant-derived organic materials pass through the soil microbial biomass at some point in time (Ryan and Aravena, 1994). Hence, the question here is to what extent the accumulation of Po under eCO_2 may account for the additional P uptake. To answer this question, further study is needed to quantify the dynamics of Po at various growth stages.

5. Conclusions

Microbial P is a main contributor to the accumulation of Po in the rhizosphere of wheat grown under eCO_2. The quantification of labelled-^{13}C flow in the plant-soil-microbe continuum in this study illustrates that eCO_2 favors photosynthetic C efflux into the rhizosphere, which stimulates microbial biomass and activity to immobilize P. Among microbial populations, bacteria are more responsive to the increased photosynthetic C flow under eCO_2. Apart from microbial P, root-derived materials may also constitute to P-enriched compounds and accumulates in the rhizosphere under eCO_2. Future work is necessary to assess how the specific species of microbes in response to eCO_2 are involved in P transformation. In addition, the spectral technology with ^{31}P-NMR would be useful to identify the organic P compounds released from plants exposed to eCO_2.

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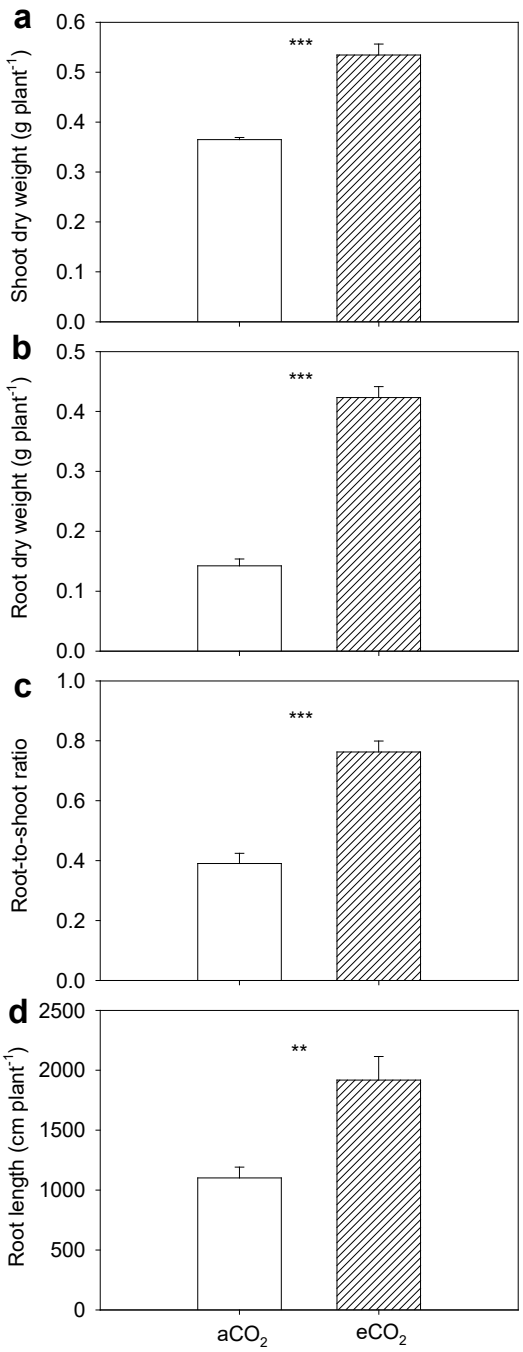

Fig. 1 The effect of CO_2 on shoot dry weight (a), root dry weight (b), and root-to-shoot weight ratio (c) of wheat after exposed to ambient (380 ppm) (aCO_2) or elevated CO_2 (800 ppm) (eCO_2) for 6 weeks. Columns are means of four replicates ± standard error. ** and *** indicate the difference between the CO_2 treatments being significant at $P < 0.01$ and $P < 0.001$, respectively.

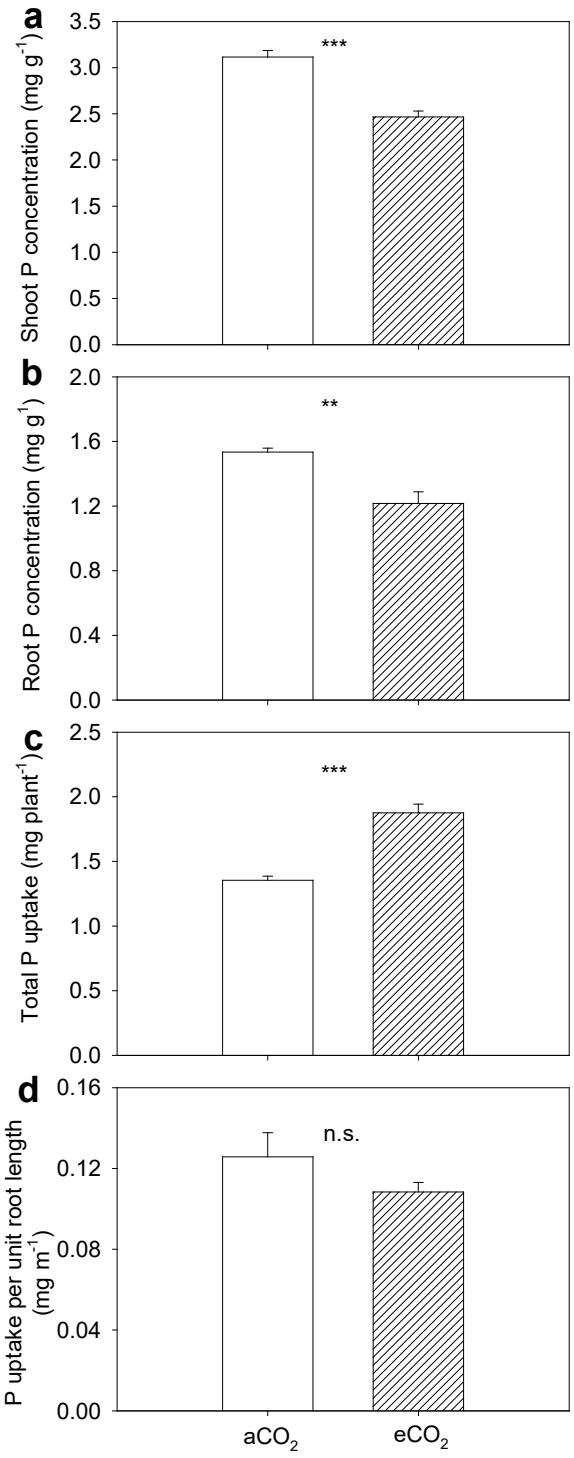

Fig. 2 The effect of CO_2 on concentrations of P in shoot (a) and roots (b), total P uptake (c) and P uptake per unit root length (d) of wheat after exposed to ambient (380 ppm) (aCO_2) or elevated CO_2 (800 ppm) (eCO_2) for 6 weeks. Columns are means of four replicates ± standard error. **, *** and n.s. indicate the difference between the CO_2 treatments being significant at $P < 0.01$, $P < 0.001$ and $P > 0.05$, respectively.

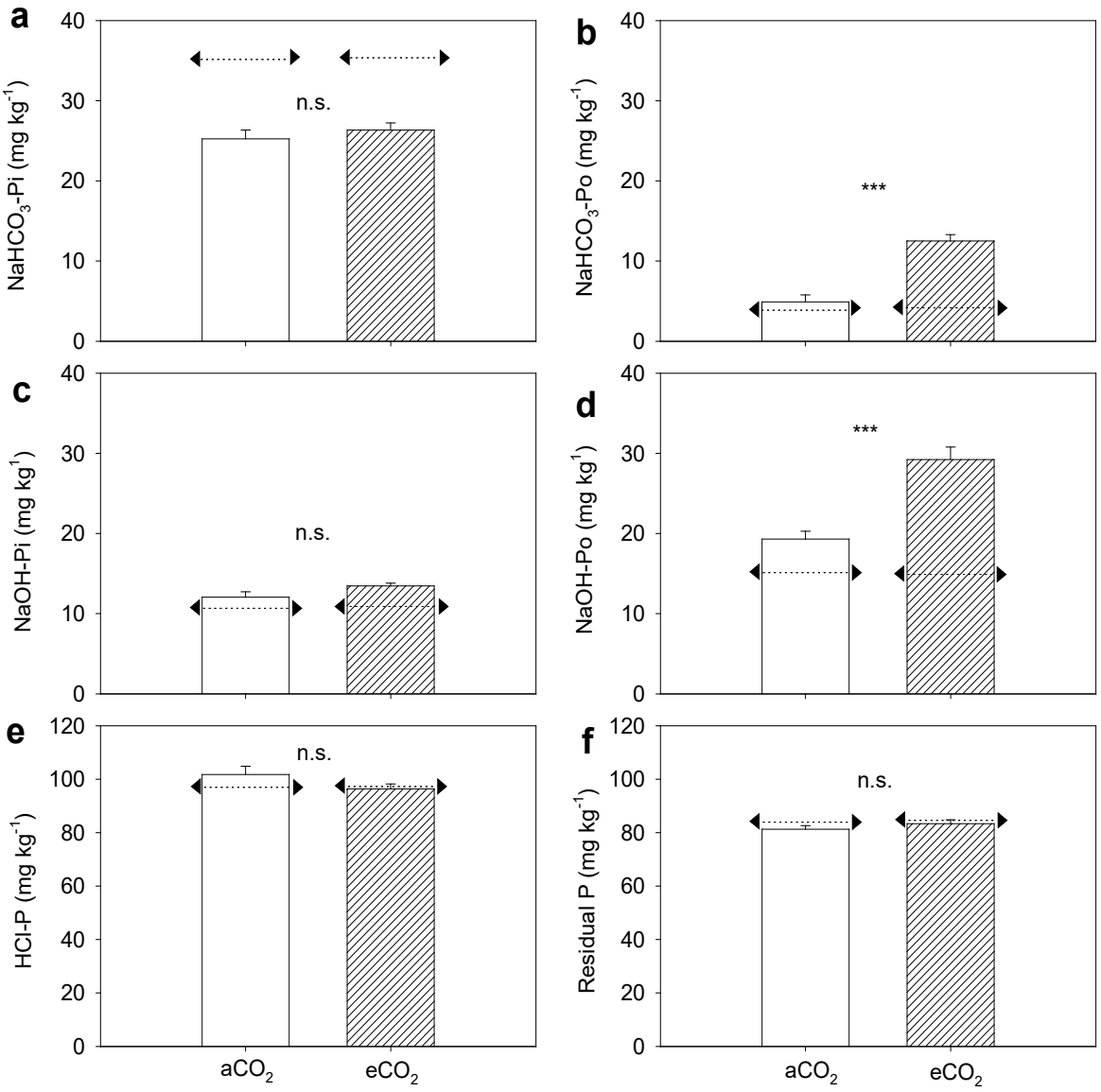

Fig. 3 The effect of CO_2 on $NaHCO_3$-extractable inorganic P (Pi) (a), and $NaHCO_3$-extractable organic P (Po) (b), NaOH-Pi (c), NaOH-Po (d), HCl-P (e) and residue P (f) in the rhizosphere of wheat after exposed to ambient (380 ppm) (aCO_2) or elevated CO_2 (800 ppm) (eCO_2) for 6 weeks. The arrow-ended short lines represent the value obtained in the no-plant control soil. Columns are means of four replicates ± standard error. *** and n.s. indicate the difference between the CO_2 treatments being significant at $P < 0.001$ and $P > 0.05$, respectively.

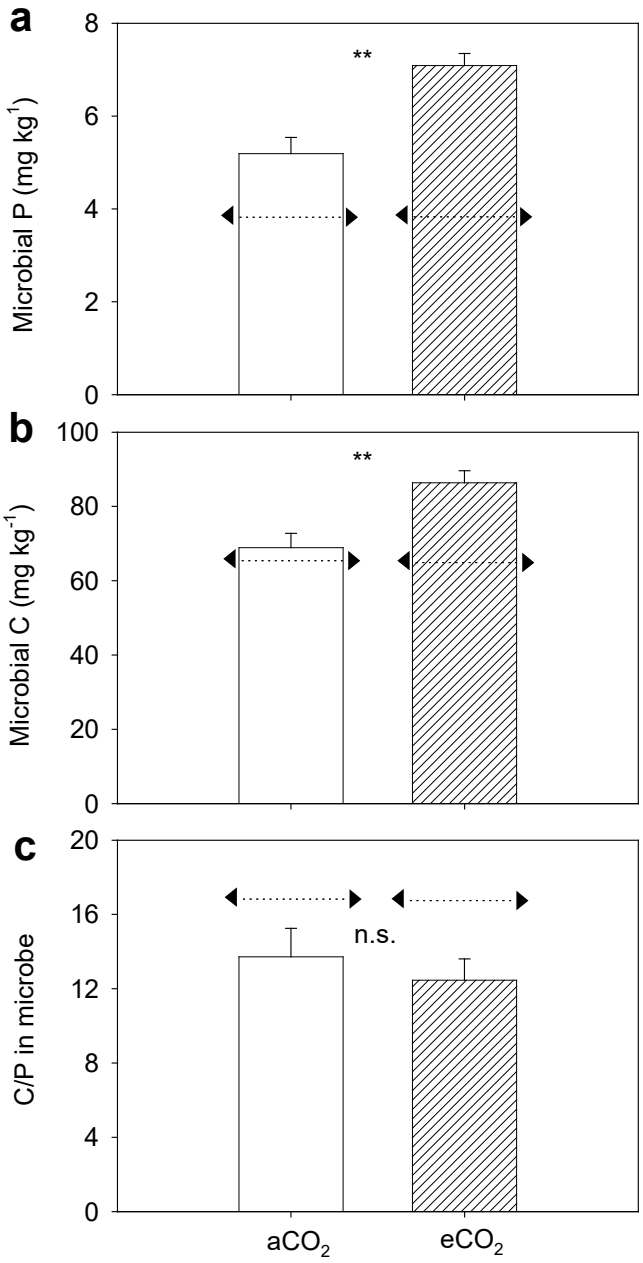

Fig. 4 The effect of CO_2 on microbial P concentration (a), microbial biomass carbon (b), and microbial C-to-P ratio (c) in the rhizosphere of wheat after exposed to ambient (380 ppm) (aCO_2) or elevated CO_2 (800 ppm) (eCO_2) for 6 weeks. The arrow-ended short lines represent the value obtained in the no-plant control soil. Columns are means of four replicates ± standard error. ** and n.s. indicate the difference between the CO_2 treatments being significant at $P < 0.01$ and $P > 0.05$, respectively.

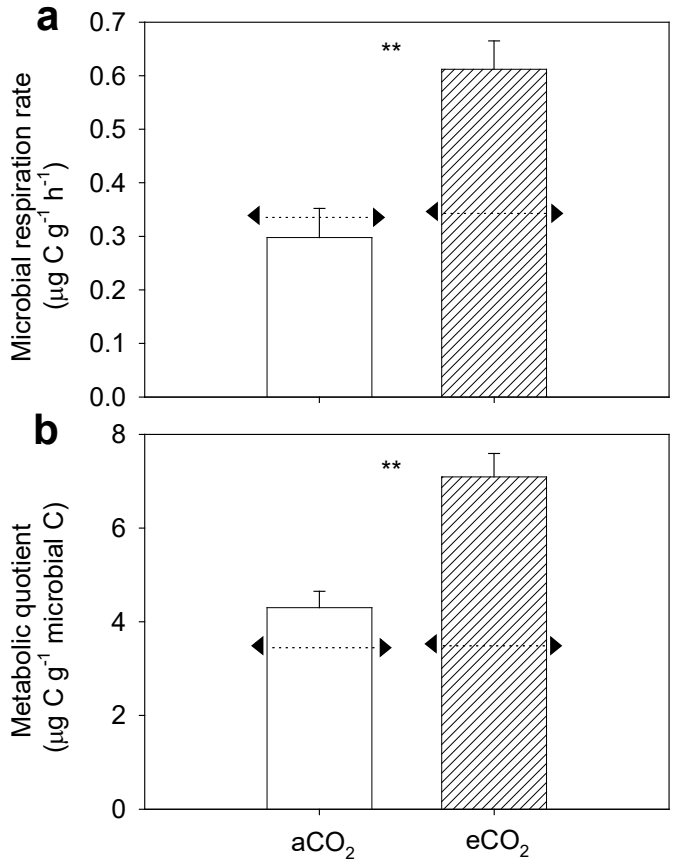

Fig. 5 The effect of CO_2 on microbial respiration rate (a) and metabolic quotient (b) in the rhizosphere of wheat after exposed to ambient (380 ppm) (aCO_2) or elevated CO_2 (800 ppm) (eCO_2) for 6 weeks. The arrow-ended short lines represent the value obtained in the no-plant control soil. Columns are means of four replicates ± standard error. **, the differences between the CO_2 treatments are significant at $P < 0.01$.

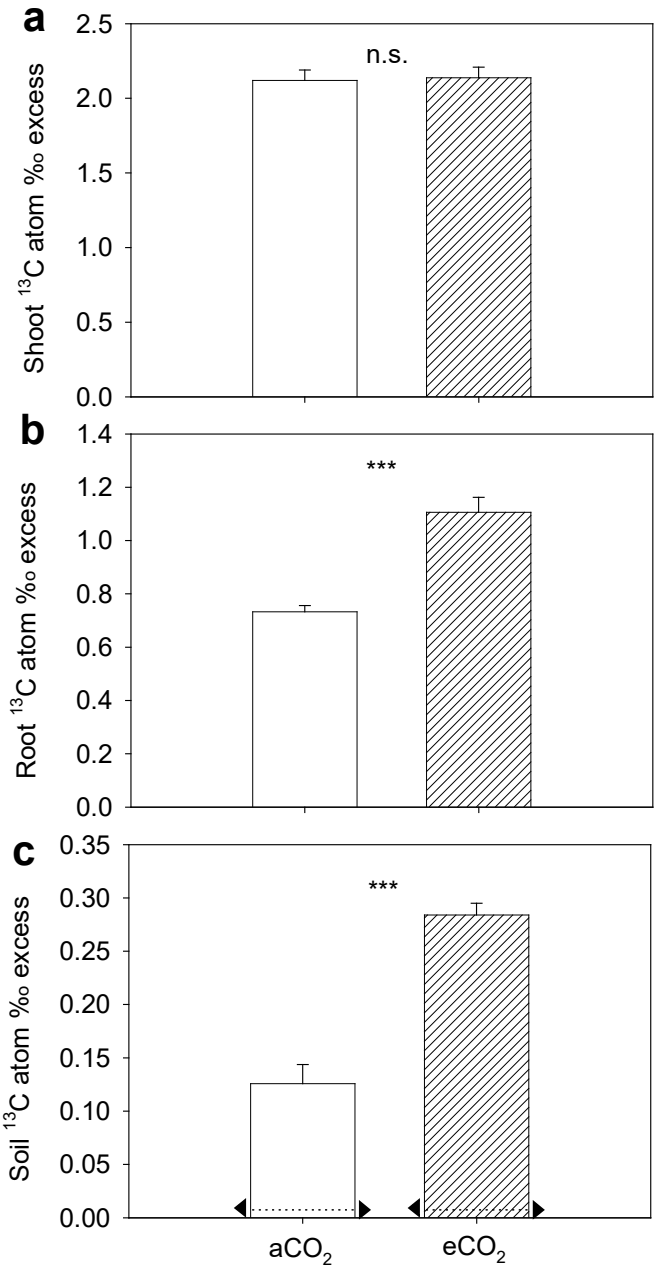

Fig. 6 The ^{13}C atom‰ excess in shoot (a), root (b) and rhizosphere soil (c) after 5-day pulse-labelling of $^{13}CO_2$. All plants were grown under ambient (380 ppm) or elevated CO_2 (800 ppm) for 6 weeks. The arrow-ended short lines represent the corresponding value in each soil treatment without growing plants. Columns are means of four replicates ± standard error. *** and n.s. indicate the difference between the CO_2 treatments being significant at $P < 0.001$ and $P > 0.05$, respectively.

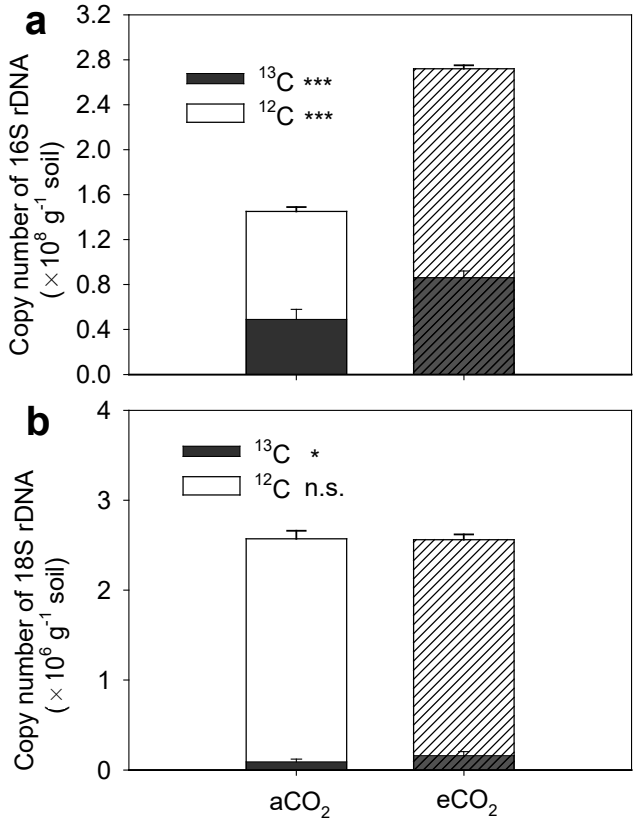

Fig. 7 16S rDNA (a) and 18S rDNA (b) copy number in ^{12}C- and ^{13}C-DNA samples that were from the rhizosphere of wheat grown under ambient (380 ppm) or elevated CO_2 (800 ppm) for 6 weeks. Columns are means of four replicates ± standard error. ***, * and n.s. indicate the difference between the CO_2 treatments being significant at $P < 0.001$, $P < 0.05$ and $P > 0.05$, respectively.