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Title:

Long-term effects of elevated CO₂ on carbon and nitrogen functional capacity of microbial communities in three contrasting soils

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Abstract

Elevated atmospheric CO₂ (eCO₂) affects soil-plant systems by stimulating plant growth, rhizosphere processes and altering the amount and quality of organic matter inputs. This study examined whether these plant-mediated processes indirectly influence the structure and function of soil microbial communities and soil carbon (C) and nitrogen (N) cycling. Surface soils (0-5 and 5-10 cm) of Calcarosol, Chromosol and Vertosol were sampled after 5 years' exposure to either ambient CO₂ (aCO₂; 390 ppm) or eCO₂ (550 ppm) using free-air CO₂ enrichment (SoilFACE). Changes in microbial community structure were not detected using automated ribosomal intergenic spacer analyses (ARISA). However, quantitative PCR of targeted organic C decomposition (*cu*, *cbh*), N mineralisation (*apr*, *npr*), nitrification (*amoB*, *amoA*, *norA*) and denitrification (*nirK*, *narG*, *nosZ*) genes showed that eCO₂ reduced the abundance of half of the functional genes in the Chromosol and Vertosol and their abundance was tightly coupled with total C and N pools. In the Chromosol, total N and C of soil (<2 mm particles) was reduced by up to 20% and was associated with enhanced microbial activity (soil respiration). Soil C was also reduced in the Vertosol (15%, 5-10 cm); however greater laccase, reduced cellulase and lower microbial activity indicated that organic matter decomposition was currently limited by N. The loss of soil organic N and C under eCO₂ was likely driven by greater N demand. This study highlighted that the indirect effects of eCO₂ on functional capacity of soil microbial communities in dryland agricultural system depended on the soil type.

1. Introduction

Increases in the carbon dioxide (CO₂) concentration in the atmosphere are expected to alter the productivity and sustainability of agricultural cropping systems. Elevated CO₂ (eCO₂) directly influences plant productivity by enhancing photosynthesis, reducing transpiration and increasing both transpiration and N-use efficiency (Morison and Gifford, 1984; Torbert et al., 2000; Ainsworth and Long, 2005; de Graaff et al., 2006; Tausz-Posch et al., 2012). Plant-induced changes in the amount, composition and distribution of organic matter inputs occur (Rogers et al., 1994) and these may indirectly affect the composition of soil microbial communities and their functional capacity associated with C and N cycling (Bardgett et al., 2008). Direct impacts of eCO₂ on soil microbes are unlikely since CO₂ concentrations in the soil are already orders of magnitude greater than those of the atmosphere. A better understanding of the long-term effects of eCO₂ on microbial communities and associated key processes, particularly N cycling and C mineralization, is required.

Changes in microbial community composition and structure under eCO₂ are highly variable (Drigo et al., 2008) and are likely due to preferential stimulation of different groups of soil organisms (Pritchard, 2011). Increases in fungal abundance and diversity have been observed in semi-arid ecosystems (Drigo et al., 2007; Lipson et al., 2014; Procter et al., 2014). A greater influence of eCO₂ on fungi than bacteria has been proposed due to the reduction in N content of plant residues (Cotrufo et al., 1998) although enhanced bacterial abundance and diversity have also been observed (Norby et al., 2001; Drissner et al., 2007). Similarly, eCO₂ increased the abundance of actinobacteria and diversity of archaea in an Australian grassland (Hayden et al., 2012) but reduced species richness and did not alter the abundance of dominant organisms in another (He et al., 2012). Nevertheless, it is important to ascertain whether changes in soil microbial communities under eCO₂ alter their functional capacity. Drissner et al. (2007) suggested that increased microbial biomass and bacterial diversity buffered the potential increase in soil C of a grassland during 9 years of eCO₂. Similarly, increased fungal abundance and C mineralization could negate additional C inputs under eCO₂ or even reduce soil fertility in the long-term (Dijkstra et al., 2005; Carney et al., 2007; Drigo et al., 2013). While eCO₂ stimulated key genes involved in the C and N cycles in soybean (He et al., 2014), little information exists for N-limited environments. Furthermore, few studies have attempted to link soil microbial and chemical properties, particularly under field conditions (He et al., 2010; He et al., 2014).

Since belowground processes are mediated by the plant, microbial responses to eCO₂ are likely to be system specific (Carrillo et al., 2014). In dryland agricultural systems, crops are grown in monoculture and soil organisms endure long dry periods in the absence of a living plant. Although some organic-N cycle processes can continue under dry conditions (Phillips et al., 2015), N availability is generally limited by low N content. We have observed increased wheat and field pea biomass under free-air CO₂ enrichment (FACE) in a semi-arid environment (Butterly et al., 2015a, b) and reduced wheat residue quality (greater C:N ratio), consistent with other studies (Norby et al., 2001). Under eCO₂, greater microbial activity may not enhance residue decomposition (Lam et al., 2014), particularly when N is limited (Hu et al., 2006). Microbial release of N from legume residues is critical for N fertility, especially in soils that have a limited capacity to supply N from soil organic matter. Microbial responses to eCO₂ are likely to depend on the soil environment. However, few studies have considered more than one soil type (Procter et al., 2014).

This study aimed to quantify the effects of eCO₂ on changes in soil C and N, and microbial biomass size and activity, community composition and functional capacity after 5 years of exposure to free-air CO₂ enrichment (FACE). To our knowledge this is the first study to examine the effects of eCO₂ on microbial process in three contrasting soil types under the same agronomic and environmental conditions. In particular, we hypothesised that (i) eCO₂

would indirectly alter microbial community composition (favour fungi over bacteria) and the abundance of functional genes due to changes in C and N inputs, (ii) soil C would remain unchanged as altered microbial communities would be able to decompose residues with a greater C:N (additional C input offset by faster C mineralization), and (iii) eCO₂ would increase the abundance of functional genes which correlated with labile C and N pools (products of decomposition).

2. Materials and methods

2.1. Field experiment design and sampling details

This study was conducted using the soil free-air carbon dioxide enrichment (SoilFACE) facility at the Department of Economic Development, Jobs, Transport & Resources (DEDTR), Horsham, Victoria, Australia (36°44'57"S, 142°06'50"E). The facility was described in detail in Butterly et al. (2015a). Briefly, intact soil mesocosms (0.3 m diameter; 1.0 m depth) of Calcarosol, Chromosol and Vertosol (Isbell, 1996) or Calcisol, Luvisol and Vertisol (WRB, 2014), representing major soil types within dryland cropping systems of South-Eastern Australia were used. The Calcarosol had 6.4 g C kg⁻¹, 0.5 g N kg⁻¹, pH 5.9, 84.5% sand 10% silt and 5.5% clay, the Chromosol 46.6 g C kg⁻¹, 4.0 g N kg⁻¹, pH 4.5, 15.9% sand 65.8% silt and 18.3% clay and the Vertosol 11.0 g C kg⁻¹, 0.8 g N kg⁻¹, pH 7.7, 12% sand 36.9% silt and 51.1% clay. Mesocosms were arranged into bunkers sunk into the ground so that the soil surface of each mesocosm was at ground level and exposed to either ambient CO₂ (aCO₂; 390 ppm) or elevated CO₂ (eCO₂; 550 ppm) concentration for 5 years/growing seasons. Each treatment was replicated four times. The FACE system used to achieve eCO₂ was outlined in Mollah et al. (2009). 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 and canola in 2013, except that canola was grown for the last two years in the Calcarosol. The facility is located in the Victorian Wimmera region characterised by a Mediterranean climate with a mean annual rainfall of 436 mm (range 544 in 2010 to 288 mm in 2012) over the experimental period. At maturity, plant shoots were cut-off at the base of the stems and transported to the laboratory. Following the removal of grain, the remaining residues were chopped (< 2 cm) and returned to their respective mesocosms in the field. Mesh netting was used to ensure that residues were not lost during the summer period.

Surface soils (0-5 cm and 5-10 cm) were sampled in September 2013. Within each replicate, a composite sample for each soil type and layer was obtained by combining nine soil samples i.e. 3 soil cores (2.5 cm OD × 5 cm long) × 3 mesocosms. Soils were transported

on ice and stored at 4°C. On the following day, soils were sieved (≤ 2 mm) and subsamples for molecular work were transferred to sterile polybags (Twirl-em, Labplas, Canada), immediately frozen using liquid-N₂ and subsequently stored at -80°C. The remaining samples were returned to 4°C overnight while the gravimetric water contents (subsamples dried at 105°C) were obtained. Overall, the study consisted of a randomised split-plot design with two CO₂ concentrations (main-plots), three soil types (sub-plot), two soil depths (sub-plots) with 4 replicates (n = 48).

2.2. Pre-incubation

A pre-incubation was performed prior to chemical and biological measurements (not involving DNA) to eliminate any confounding effects due to spatial and soil-type differences in soil water content. Soils were adjusted to 60 % field capacity (θ_g) using reverse osmosis (RO) H₂O where θ_g was 0.12, 0.46 and 0.44 g g⁻¹ for Calcarosol, Chromosol and Vertosol, respectively and incubated at 25°C for 10 days. Bags were opened periodically to allow gas exchange with ambient air. At the end of the pre-incubation period, soils were destructively sampled for soil respiration, microbial biomass C and N and inorganic N. All other analyses were performed using air-dried soil.

2.3. Soil respiration

Soil respiration (SR) and substrate-induced respiration (SIR) were quantified using air-tight incubation jars (Butterly et al., 2009) and traditional alkali absorption (Zibilske, 1994). Briefly, 5 g dry weight equivalent (DW) of each soil was placed into individual jars (Ball Half Pint, Jarden Corporation, USA) with two vials containing 8 ml RO H₂O to maintain headspace humidity or 3 ml of 0.5 M NaOH to trap CO₂. Two sets of soil cores receiving the same volume (70% θ_g) of either RO H₂O or glucose solution (600 μ g C g⁻¹ soil) were included. A set of jars with no soil was included as a control. Jars were incubated at 25°C in the dark for 16 days. The jars were gently agitated daily to maximise trapping efficiency and traps were exchanged with new ones at 2 days. Soil and substrate-induced respiration (μ g CO₂-C g⁻¹ soil) were estimated by titrating 1.5 ml of each trap and 1.72 M BaCl₂ (1:1) with 0.25 N HCl and phenolphthalein indicator (1% w/v in ethanol) using a digital burette (Brand Titrette, Germany).

2.4. Microbial biomass C and N and inorganic N

Microbial biomass C (MBC) and N (MBN) were quantified using 24-h fumigation-extraction according to (Vance et al., 1987) but with the following modifications. Soil (7 g DW) was extracted with 28 ml 0.5 M K₂SO₄ and shaken end-over-end for 1 h before centrifugation at 4000-rev min⁻¹ (2000 g) for 6 min. Extracts were passed through Whatman #42 filter papers using vacuum and stored at -20°C until analysis.

Organic C concentrations in fumigated and non-fumigated extracts were determined using wet-oxidation (Vance et al., 1987) as modified by (Heanes, 1984). Briefly, 2 ml of extract, 2 ml of 1 N K₂Cr₂O₇ and 4 ml of 98% H₂SO₄ were reacted at 130°C for 40 min. Solutions with known concentrations of C₁₂H₂₂O₁₁ were included as standards. After cooling, the C concentration in digests was determined spectrophotometrically at 600 nm. The C contained within digested non-fumigated samples was denoted extractable organic C (EOC). Microbial biomass C (MBC) was estimated as the difference between fumigated and non-fumigated samples using a k_{EC} of 0.37 (Sparling and Zhu, 1993; Joergensen, 1996).

Total N contained within fumigated and non-fumigated extracts was determined using the wet-oxidation method of Cabrera and Beare (1993). Specifically, 2.5 ml of extract and digestion mix (50 g K₂S₂O₈ and 30 g H₃BO₄ in 100 ml of 3.75 M NaOH adjusted to 1 l with H₂O) (1:1) were autoclaved (121°C, 104 kPa) for 30 min and stored at 4°C until analysis. Solutions with known concentrations of CO(NH₂)₂ were included as controls. The N (NH₄⁺ + NO₃⁻) concentration of extracts was determined using a flow injection analyser (Lachat QuickChem 8500 Series II, USA). The N contained within digested non-fumigated samples was denoted extractable organic N (EON). Microbial biomass N (MBN) was estimated as the difference between fumigated and non-fumigated samples using a k_{EN} of 0.54 (Brookes et al., 1985). Inorganic N (N_i) (NH₄⁺ + NO₃⁻) in non-fumigated, non-digested extracts was also determined to indicate the pool of available N.

2.5. Total C, total N, pH and texture

Total C (Ct) and N (Nt) concentration of soils were determined by dry combustion (Perkin Elmer 2400 Series II, USA) following manual grinding and homogenisation using a mortar and pestle. Soil pH_{CaCl₂} was determined using a pH meter (Thermo Orion 720A+, Beverly, MA, USA) following extraction of 5 g air-dried soil with 0.01 M CaCl₂ (1:5) by shaking end-over-end for 1 h and centrifugation at 492g for 10 min. Soil texture was assessed following the dispersion of soil (~10 g) in 800 ml RO H₂O containing 10 ml Na₆P₅O₁₈ (1% w/w) using a Laser Particle Size analyser (Malvern Mastersizer 2000, Worcestershire, UK).

2.6. Microbial community composition

Microbial community composition was assessed using automatic ribosomal intergenetic spacer analysis (ARISA). Firstly, total community DNA was extracted from 250 mg (fresh weight present at the time of field sampling) soil using the PowerSoil DNA isolation kit (MoBio, Calsbad, USA). Final DNA concentrations were estimated using a P-Class NanoPhotometer (Implen, Muich, Germany) and normalised to 68 ng DNA μl^{-1} using sterile Milli-Q H₂O. ARISA-PCR was performed using a 1- μl aliquot of each normalised sample in a 20 μl reaction volume containing 4 μl Qiagen Q solution, 2 μl Qiagen TopTaq 10 $\times$ buffer, 1 μl Qiagen 10 mM dNPT, 1.2 μl 25 mM MgCl₂, 0.1 μl Qiagen TopTaq DNA polymerase and 1 μl appropriate forward and reverse primers (10 mM). Bacterial communities were amplified using universal bacterial primers [16S-1392F, 23S-125R; (Fisher and Triplett, 1999)] according to Kovacs et al. (2010) using a TProfessional TRIO thermocycler (Biometra, Goettingen, Germany). Fungal communities were amplified using universal fungal primers [ITS1F, ITS4R; (White et al., 1990; Gardes and Bruns, 1993)] according to Lee and Taylor (1992) using a CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, USA). Primers 16S-1392F and ITS4R were labelled with the fluorescent marker 6-carboxyfluorescein at the 5' end. Fragments were separated on an Applied Biosystems 3730 DNA analyser and data were analysed using GeneMapper 4 software (Life Technologies, Foster City, USA) in a commercial laboratory (Australian Genome Research Facility, Melbourne, Australia). Fragments of 50-1200 and 50-1000 bp for bacteria and fungi, respectively were binned and normalized using automatic and interactive binning scripts (Ramette, 2009) in a stepwise procedure using R (The R Foundation for Statistical Computing, Boston, USA). Bin sizes of 2 and 4 were used for 0-700 and 701-1200 bp ranges, respectively for both bacteria and fungi.

2.7. Microbial community function

Microbial community function was assessed by quantitative PCR of key genes involved in soil organic matter decomposition and N transformation. Briefly, DNA was extracted in duplicate from field moist soil as previously described. DNA quality and quantity was determined spectrophotometrically (NanoDrop 2000, ThermoScientific), and then replicate extractions were pooled and re-quantified. Quantified organic C decomposition genes were laccase [*cu1Af/2r*, Kellner et al. (2007)] and cellobiohydrolase [*cbhIf/r*, Edwards et al. (2008)], mineralisation genes were alkaline and neutral protease [*aprI/r* and *nprI/r*, Bach et al. (2001)], nitrification genes were bacterial and archaeal ammonia monooxygenase [*amoA1f/2r*, Rotthauwe et al. (1997) and Arch-*amoA*f/r, Wuchter et al. (2006), respectively] and nitrite oxidoreductase [*norAf1/r1*, Poly et al. (2008)], and denitrification genes were nitrate reductase [*narGf/r*, Bru et al. (2007)], nitrite reductase [*nirK* 583f/909r, Yan et al. (2003)] and

nitrous oxide reductase [*nosZ2f/2r*, Henry et al. (2006)]. Quantitative PCRs were performed in a 10- μ l-reaction volume containing 10 ng DNA, using a Bio-Rad CFX 384 Real-Time PCR Detection system (Bio-Rad, Gladesville, NSW, Australia). For the first 5 genes, primer concentrations, reagents and cycling conditions were as described in Phillips et al. (2015); remaining genes were amplified using Bioline SensiFAST (*nosZ*) or Bio-Rad SsoAdvanced (Arch-*amoA*, *norA*, *narG*, *nirK*) mastermixes and 0.5 μ M each primer, for 40 cycles of 95°C for 5 sec and 60°C for 30 sec, with an initial denaturation step of 95°C for 3 min and a final melt curve from 55°C to 95°C. For each gene, a minimum of two assays was run on a single plate in a single run (Smith et al., 2006). Absolute quantification was performed by comparison against calibration standards [as described in Phillips et al. (2015)] with a linear range from 2 - 2×10^7 copies.

2.8. Statistical analyses

A three-way analysis of variance (ANOVA) using a split-split-plot design was used to test the effects of CO₂ history (main-plot), soil type (sub-plot 1) and soil depth (sub-plot 2) on soil chemical properties, soil respiration and functional gene abundance using GenStat (VSN International, Hemel Hempstead, UK). Ct and Nt were log(x) transformed before analysis. Differences between means were tested using the least significant difference (LSD) test at $P=0.05$.

The effects of CO₂ history $\times$ soil type $\times$ soil depth on bacterial and fungal community structure were determined by permutational multivariate analysis of variance (PERMANOVA) using PRIMER 6 (PRIMER-E, Lutton, UK) following square-root transformation. Pair-wise distances were calculated using Bray-Curtis and communities were represented using non-metric multidimensional scaling (MDS) and two-dimensional mapping.

Relationships between gene abundance and soil properties were assessed by correlation analysis, using Pearson's *r* or Spearman's *rho* for normal or non-normal data, respectively (normality assessed by Shapiro-Wilk's *W*) with PAST v2.17 software (Hammer et al., 2001). All significant correlations were visually assessed to ensure outliers did not influence results. Only those measures that were continuous across the two soil depths were assessed. N_i, EON, and soil respiration, which were highly depth-stratified, were excluded from analysis.

3. Results

3.1. Carbon and nitrogen in soil and microbial biomass

Five years' exposure to elevated CO₂ altered Ct and Nt but this effect depended on the soil type (Figure 1). Significant CO₂ × soil interactions for Ct ($P=0.015$) and Nt ($P=0.050$) were observed. Elevated CO₂ decreased Ct and Nt by 20 % in the 5-10 cm layer of the Chromosol (Figure 1b,e), and decreased Ct by 15 % in the 5-10 cm layer of the Vertosol (Figure 1c,f). The C:N ratio was not affected by the treatments, except for a decreased C:N in the 5-10 cm layer of the Vertosol (Figure 1g,h,i).

Microbial biomass C and N, and labile C and N pools were strongly affected by soil type and depth but less affected by CO₂ history (Table 1). Elevated CO₂ decreased MBC by 65 and 104 mg C kg⁻¹ soil in the Chromosol and Vertosol surface (0-5 cm) soil layers, respectively, but did not affect it in the deeper (5-10 cm) soil layers and in both layers of the Calcarosol, leading to significant CO₂ × soil ($P=0.001$) and CO₂ × depth ($P=0.012$) interactions. Inorganic N (N_i) showed a CO₂ × depth ($P=0.012$) interaction, whereby eCO₂ decreased N_i concentrations in the 0-5 cm soil layers. However, the decrease in N_i under eCO₂ was only significant for the Chromosol (by 10 mg N kg⁻¹) since the other two soils had lower initial N_i concentrations. Similarly, CO₂ significantly ($P=0.012$) decreased EON by 4 mg N kg⁻¹ soil in the 0-5 cm layer of the Vertosol.

In general, MBN and EOC in the 0-5 cm soil layer and MBC, MBN, EOC, EON and N_i in the 5-10 cm layer were primarily influenced by soil type and depth (Table 1), and the size of these C and N pools were in the order of Chromosol > Vertosol > Calcarosol. However, compared with Chromosol and Calcarosol EON and N_i concentrations in the Vertosol were low considering the Nt and MBN content (Figure 1, Table 1). As expected, the 0-5 cm soil layer had greater concentrations of microbial biomass and labile C and N than the 5-10 cm layer.

3.1. Soil and substrate-induced respiration

The effects of CO₂ history on SR depended on the soil type and depth (Table 1). In general, SR was greater in soils exposed to eCO₂, although this was only significant in the 0-5 cm layer of Calcarosol. In addition, SR was lower in the 5-10 cm layer compared with the 0-5 cm layer, except for in the Vertosol. In 0-5 cm layer, SR in the Chromosol approximately doubled that of other two soils (Table 1). In 5-10 cm layer, the relative differences in SR between the soils were small, despite large differences in Ct and Nt or MBC (Figure 1, Table 1). In contrast, the main effect of CO₂ history on SIR was not significant nor was its interactions with soil and depth (Table 1). A significant soil × depth ($P<0.001$) interaction was observed whereby the relative magnitude of SIR was in the order of Chromosol > Vertisol > Calcarosol, and for each soil SIR was lower in 5-10 cm than the 0-5 cm soil layers.

3.2. Microbial community fingerprinting using ARISA

Soil microbial community fingerprinting using ARISA showed that community composition differed significantly between soil types (Figure 2). Bacterial communities (Figure 2a) clustered more tightly within soil than did fungal communities (Figure 2b), indicating greater differentiation of bacterial species than fungi in these agricultural soils. However, CO₂ history did not significantly alter the dominant members of the bacterial or fungal microbial community. For bacteria, the number of unique (aCO₂ or eCO₂) and shared (aCO₂ and eCO₂) operational taxonomic units (OTU's) were similar, except in the Chromosol where unique OTU's increased from 10 (aCO₂) to 15 (eCO₂) with no change in their percentage contribution to total sample fluorescence (Fig. S1). For fungi, unique OTU's increased from 1 (aCO₂) to 6 (eCO₂) in the Chromosol but decreased from 5 (aCO₂) to 1 (eCO₂) in the Vertosol, with respective changes in percentage contribution to total sample fluorescence. Overall, 192 OTU's were identified for bacteria and 96 for fungi.

3.3. Functional gene abundance

Functional gene abundance (Table 2: copies g⁻¹ soil and Table S1: copies ng⁻¹ DNA) was greatly influenced by soil type and depth. Archaeal *amoA*, however, had equivalent abundance in all soils, and cellulase, neutral peptidase, bacterial *amoA*, nitrite reductase and nitrous oxide reductase were comparable in the Vertosol and Chromosol. Notably, laccase was the highest in the Vertosol, 2.8 and 5.3 times greater than the Chromosol and Calcarosol, respectively. Given the large influence of soil and depth, no main effects of CO₂ were observed and CO₂ × depth interactions were only observed for laccase and nitrate reductase genes (Table 2) with an apparent effect of CO₂ for neutral peptidase ($P=0.055$). Nevertheless, post-hoc tests show that eCO₂ decreased the abundance of ~50% of the functional genes in Chromosol and Vertosol (Table 2). For example, laccase, cellulase and neutral peptidase in the Vertosol as well as a number of genes involved in inorganic N cycling in both Chromosol and Vertosol were fewer under eCO₂. In the Calcarosol, the relative abundance of nitrate reductase and nitrite reductase were greater under eCO₂ than aCO₂ in the 0-5-cm layer.

3.4. Relationships between functional genes and soil chemistry

The relative abundances of functional genes generally increased with increasing fertility (Ct, Nt, MBC, MBN, EOC, EON), i.e. Chromosol > Vertosol > Calcarosol and 0-5 cm > 5-10 cm (Table 3). Under eCO₂, there was a strong relationship between total

extractable DNA and organic matter, which resulted in highly significant correlations of all functional genes (copies g⁻¹ soil) with Ct and Nt (Table S2). Although this indicates a closer relationship between microbial functions and the substrates and products of the reactions they catalyse under eCO₂, it also reflects the inherent differences in soil fertility. More importantly, when specific functional gene abundance (copies ng⁻¹ DNA) was assessed (i.e. disregarding changes in microbial biomass), stronger correlations were still observed under eCO₂ than aCO₂ (Table 3). This finding highlights specific changes in the functional capacity of the soil microbial communities under eCO₂. For example, in the Calcarosol, Ct and Nt correlated with archaeal *amoA* under aCO₂ but with bacterial *amoA* and nitrite oxidoreductase under eCO₂. In fact, stronger correlations between bacterial *amoA* and Ct and Nt were observed in all soils under eCO₂ than under aCO₂. Furthermore, under eCO₂ laccase correlated positively with Ct, Nt and EON in the Chromosol. Overall, the relative changes in the specific abundance of functional genes and soil C and N pools properties indicate greater coupling of C and N cycling under eCO₂ (Table 3).

The relationships between specific gene abundance and soil chemical properties were assessed by NMDS ordination (Figure 3). The vector overlay shows the relative influence of each functional gene in the separation of the soils and is consistent with that previously described (Table 3). Soil chemical data showed significant interactions between CO₂ and soil type in both soil layers (Figures 3a,b). In contrast, overall patterns of functional gene abundance were significantly different between soil types, but were not affected by CO₂ history (Figure 3b,d). However, under eCO₂ functional genes generally cluster more tightly. The matrices show a greater resemblance of soil chemical data in the Chromosol compared with the two other soils.

4. Discussion

This study reported the longer-term (>5 years) effects of eCO₂ (FACE) on the size, activity, community composition and functional capacity of microbes and associated C and N cycles in three contrasting agricultural soils under the same agronomic and environmental conditions. Using a targeted sequence of C and N functional genes we showed that the response of microbial communities of soil-plant systems after 5 years of FACE was predominantly soil-type specific.

4.1. Effect of eCO₂ on microbial biomass size and activity

The response of the microbial biomass to eCO₂ was governed by soil type and depth (Table 1). The initial MBC contents of the three soils reflected the relative C contents and soil

textures. The effect of eCO₂ on microbial biomass, however appeared to be positive for the relatively infertile Calcarosol and negative for Chromosol and Vertosol soils. Increases in microbial biomass under eCO₂ have been associated with greater C inputs (de Graaff et al., 2006; Drissner et al., 2007; Cheng et al., 2011). In a 6-week glasshouse study using a similar Calcarosol planted with either wheat or field pea, eCO₂ increased microbial biomass (Jin et al., 2013). However, eCO₂ did not affect microbial biomass in grassland (Hungate et al., 2000; Soe et al., 2004; Dijkstra et al., 2005; Kandeler et al., 2006; Reinsch et al., 2013), heath (Haugwitz et al., 2014) and cropping soils (Weigel et al., 2005).

There are two possible explanations for the decreased microbial biomass in Chromosol and Vertosol under eCO₂. Firstly, the large reduction in MBC and concomitant increase in MBC:MBN in the Vertosol indicates competition between microbes for available N. The Vertosol had the lowest labile (EON) and available (N_i) N among the three soils. Furthermore, Jin et al. (2013) showed that eCO₂ only enhanced the microbial biomass in this Vertosol when growing a legume but not wheat. Decreased N availability under eCO₂ limits microbial growth rates (Blagodatskaya et al., 2010) and thus microbial biomass, particularly during the later years. Secondly, a large component of the microbial biomass in the Chromosol and Vertosol may have been functionally inactive under aCO₂ and were subsequently lost under eCO₂ due to their inability to compete with other microbes. This ‘functional redundancy’ of soil microbes is well established (Allison and Martiny, 2008) and is supported by the fact that soil respiration remained unchanged or changed very little despite large reductions in microbial biomass.

Microbial activity was greater under eCO₂ in the topsoil of the Calcarosol (0-5 cm layer) during the 16-day study as indicated by greater soil respiration (Table 1) and is consistent with most studies (Pendall et al., 2001; de Graaff et al., 2004). Furthermore, eCO₂ increased (20%, Chromosol) and decreased (37%, Vertosol) soil respiration over a 96-day period (Unpublished data), confirming that the apparent differences in soil respiration under eCO₂ observed here were amplified over time. The increased activity of the microbial biomass under eCO₂ is likely to be due to greater labile C availability (Allard et al., 2006) or an enhanced ability of soil microbes to utilise existing soil C pools (Carney et al., 2007; Reinsch et al., 2013). In the current study, we speculate that microbial activity was primarily determined by C substrate availability since substrate-induced respiration (Table 1) was not affected by eCO₂. Therefore, lower microbial activity in the Vertosol under eCO₂ than aCO₂ indicates that the generation of C substrate via decomposition was limited.

4.2. Effect of eCO₂ on microbial community composition

Distinct bacterial and fungal communities were observed within each soil type as expected (Xu et al., 2013) however, eCO₂ did not alter microbial community composition or the relative abundances of bacteria and fungi (Figure 2). Greater C inputs via crops combined with an increase in C:N ratio of non-legume residues were expected to favour fungi over bacteria as previously observed in N-limited grasslands (Hu et al., 2001; He et al., 2012), shrub (Carney et al., 2007; Lipson et al., 2014) and fertilised cropping systems (He et al., 2014). In particular, the central role of mycorrhizal fungi for C cycling is commonly intensified under eCO₂ (Denef et al., 2007; Drigo et al., 2013; Fang et al., 2015). However, the responses of soil microbial communities to eCO₂ differ greatly including: increased bacteria but not fungi (He et al., 2010), increased archaeal and fungal diversity and Actinobacterial abundance with no change in bacterial and fungal abundance (Hayden et al., 2012) and increased fungi:bacteria and fungi:gram-ve bacteria ratios despite changes at the community level (Guenet et al., 2012).

Similar to the current study, significant effects of eCO₂ on microbial community composition are often not detected using whole community DNA (ARISA, T-RFLP, DDGE) or fatty acid (PLFA) fingerprinting techniques (Ebersberger et al., 2004; Chung et al., 2007; Gutknecht et al., 2012). Combined fingerprinting and metagenomic approaches has had greater success (Hayden et al., 2012; He et al., 2012) and reveal that changes in composition of the most abundant organisms occurred at class or lower taxonomic levels than phylum (He et al., 2012). Similarly, rRNA gene sequencing showed that eCO₂ increased fungal diversity at multiple levels (class to genus) and across major lineages but with no involvement of specific fungal groups (Lipson et al., 2014). Fungi were less dissimilar across three contrasting agricultural soils than bacteria as indicated by ordination clusters (Figure 2). Quantifying microbial responses to eCO₂ will require high-resolution molecular tools combined with novel data processing techniques (Tu et al., 2015). In addition, genomic based approaches should utilise RNA rather than DNA to distinguish between active organisms and the rest of the microbial community (Blagodatskaya and Kuzyakov, 2013).

Microbial community responses to eCO₂ in cropping soils are expected to be inherently different from other ecosystems. Since responses to eCO₂ are positively related to plant species richness (Dijkstra et al., 2005; Chung et al., 2007), the relative effect of eCO₂ on agricultural systems based on monoculture crop rotations may be quantitatively smaller and take much longer to manifest. In dryland agricultural systems only a single crop is grown each year and soil microbes endure long 'fallow' periods of desiccation and heat in the absence of a living plant. Cheng et al. (2011) showed that changes in microbial biomass and community structure under FACE in a wheat-soybean rotation were driven by legume productivity and were not detectable in the first 3 years. It is not known whether eCO₂ effects on microbial communities were apparent in other crop phases although cycles of net N

deposition (field pea) and net N utilisation (canola and wheat) could have had positive and negative effects on soil microbes, respectively. Nevertheless, the combined effects of 5 years of FACE on soil microbial community structures were not detectable in the current study.

4.3. Effect of eCO₂ on the abundance of functional genes

To our knowledge, this is the first study to report decreases in functional gene abundance from soils exposed to eCO₂ under field (FACE) conditions. Most studies report increases in C (Chung et al., 2007), and C and N cycling genes under eCO₂ (He et al., 2014) including labile C (starch and cellulose) but not recalcitrant C (lignin) genes under grassland (He et al., 2010; Xu et al., 2013) or both in a wheat-soybean cropping rotation (Fang et al., 2015). Nevertheless, some studies have reported no effect of eCO₂ on the abundance of C (Guenet et al., 2012) and N cycle enzymes, specifically denitrification genes *amoA* and *nosZ* (Pereira et al., 2013; He et al., 2014). While Tu et al. (2015) showed that enhanced ammonification occurred with the reassembly of fungal communities, few studies have investigated the effects of eCO₂ on N cycle processes, especially in dryland cropping systems.

The response of soil microbial species and their functions to eCO₂ is commonly soil-type specific (Procter et al., 2014). In the current study changes in functional gene abundance under eCO₂ were proportional to changes in microbial biomass. In contrast, enzyme regulation (production and activity) rather than microbial abundance was shown to determine enzyme abundance under eCO₂ (Kandeler et al., 2006). The large reduction in functional genes between 0-5 and 5-10 cm, consistent with other studies (Kandeler et al., 2006; Marhan et al., 2011; He et al., 2014), highlights the importance of including different depths of this resolution. Although a greater influence of CO₂ than depth on enzyme abundance can occur in more fertile soils (Xiong et al., 2015).

Under eCO₂ total (gene copies g soil⁻¹) and specific (gene copies ng DNA⁻¹) functional gene abundances were highly coupled with Ct and Nt (Figure S1) and Ct, Nt and EON (Table 3), respectively. While other studies have shown that microbial communities (Deng et al., 2012) and specific changes in richness, composition and structure (He et al., 2012) were related to soil properties in FACE studies, few have considered function. However, correlations with soil properties are likely to be highly system specific. For example, a range of functional genes were correlated with Ct and Nt in maize (Xiong et al., 2015) but *narG* was not correlated with soil C and N in irrigated canola (Marhan et al., 2011) both grown under FACE. In the current study, Vertosol had more laccase than cellobiohydrolase, much lower specific cellulase abundance than the two other soils, and neither were correlated with soil C and N pools. This coupling of Ct and Nt with DNA based gene abundance could indicate that the release of organic N from soil organic matter and crop

residues is an important bottleneck for N cycling in this soil, rather than N mineralization. A N limitation could be the reason for reduced microbial activity under eCO₂. In contrast, laccase, cellulase and both peptidase genes were highly correlated with Ct and Nt in the Chromosol, and enhanced decomposition under eCO₂ to supply N may be responsible for the loss of Ct and Nt in this soil. Currently, we have investigated the genetic potential of the entire community but RNA or protein based techniques would provide more specific information regarding functionally active community members.

4.4. Effect of eCO₂ on soil C balance

The changes in soil C pools observed in the current study were not expected, particularly after only 5 years of FACE. Although only significant in the 5-10 cm layer of the Chromosol and Vertosol, our results indicate that Ct in the 0-5 cm layer of these soils may also decline under eCO₂ over a longer period. While eCO₂ enhances net primary productivity, additional C produced under eCO₂ is mostly labile and does not contribute to the long-term C storage (Dijkstra et al., 2005; Jastrow et al., 2005; Kandeler et al., 2006). Instead most studies report no change (de Graaff et al., 2004; Drissner et al., 2007) or small declines in soil C (Carney et al., 2007; Nie et al., 2013). Elevated CO₂-induced changes in microbial communities and their function (Fontaine et al., 2004; Carney et al., 2007; Nie et al., 2013; Pendall et al., 2013) can enhance the decomposition of soil C (van Groenigen et al., 2014) via increases in lignin degrading fungi (Carney et al., 2007) and arbuscular mycorrhizal fungi (Cheng et al., 2012). In addition, increased root surface area, rhizodeposition, greater periods of adequate rhizosphere moisture or the extension of the growing season, can facilitate soil C decomposition by microbes (Allard et al., 2006; Paterson et al., 2008; Xiong et al., 2015). Interestingly, eCO₂ has been shown to enhance the degradation of existing SOC within aggregates (Dorodnikov et al., 2011). Greater losses of C are likely to be exhibited in soils with high N contents (Carney et al., 2007; He et al., 2014), such as the Chromosol in the current study.

The reduction in total C of soil (<2 mm particles) does not indicate a net loss of C in the soil-plant system under eCO₂, since plant root and buried (>2 mm fragments) and above-ground crop residues are excluded. Less C in the <2-mm fraction may indicate a net loss of C from the soil-plant system via rhizosphere effects or C priming. Alternatively, it could signify that the replenishment of the <2-mm fraction from above-ground litter and buried residues (> 2 mm) has been interrupted. Increased C:N ratio of non-legume residues has been reported at this site (Butterly et al., 2015a), although the implications for C and N cycling are not clear. Furthermore, while greater microbial activity under eCO₂ in the Chromosol is consistent with enhanced C mineralization and C loss, microbial activity in the Vertosol was lower under

eCO₂ than aCO₂. It is possible that microbial activity and C mineralization were initially high in the Vertosol (Lam et al., 2014) but declined towards the end of the 5-year study due to limited N availability. This progressive N limitation can reduce labile C inputs and limit microbial activity (Hu et al., 2001; Luo et al., 2004).

The current study only examined the effects of eCO₂ on microbial processes during a single season and only under canola. It is not clear whether individual crops or the entire cropping rotation contributed to the changes in soil C observed. Facilities such as SoilFACE that utilise medium-size mesocosms have experimental and economic advantages, particularly making it feasible to investigate multiple crops simultaneously within each growing season. However, it should be acknowledged that these free-draining (non-suction) lysimeters could be an important trade-off over larger monoliths. For wheat and barley at least, crop growth and water extraction were comparable between plants grown in mesocosms and in the surrounding field (Nuttall et al., 2005; Nuttall et al., 2008) but this would need to be validated for other crop species. Furthermore, mesocosms that drain only by gravimetric forces, i.e. have no suction at the base, may not be appropriate in higher rainfall environments or situations where plants are not present and/or continually utilising rainfall. In the current study, mesocosms rarely had continuous columns of water due to the low and intermittent rainfall and are thought to be adequate to investigate plant-soil responses to eCO₂ within this environment.

4.5. Conclusions

Distinct chemical and biological responses of three contrasting soil types to eCO₂ were observed under the same agronomic management and environmental conditions. In a dryland agricultural system, the abundance of key organic C decomposition, N mineralisation, nitrification and denitrification functional genes were largely unaffected in a Calcarosol, but ~50% were reduced by eCO₂ in a Chromosol and Vertosol and were tightly coupled with organic substrate (Ct and Nt) pools. Soil C (<2 mm) in the 5-10 cm layer of Chromosol and Vertosol was reduced after 5 years of FACE but not affected in the less fertile Calcarosol. Thus, eCO₂ significantly modified C and N cycling albeit without changes in microbial community composition using a standard fingerprinting approach. Greater N demand under eCO₂ likely facilitated this acceleration of soil organic C and N mineralization. For the Vertosol, it appears that an initial stimulation of microbial decomposition under eCO₂ (15% loss in Ct) had since been constrained by N availability, as indicated by increased laccase abundance. This loss of Ct could indicate that soil C replenishment via crop residue decomposition is interrupted under eCO₂. Further investigation is required to ascertain the temporal and seasonal changes in microbial communities and their capacity to perform key C

and N cycle processes, particularly under different crop species. As different soil types may exhibit or develop various bottlenecks in C and N cycling under eCO₂ greater attention to belowground processes combined with crop growth and yield in different soil-plant systems is required in the future.

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Table 1

Table 1 Microbial biomass C (MBC) and N (MBN), extractable organic C (EOC) and N (EON), inorganic N (Ni), soil respiration (SR) and substrate-induced respiration (SIR) in 0-5 and 5-10 cm layers of Calcarosol, Chromosol and Vertosol soils under ambient CO₂ (aCO₂, 390 ppm) and elevated CO₂ (eCO₂, 550 ppm) for 5 years. For each parameter, means with the same letter are not significantly different at $P \leq 0.05$.

Soil & depth (cm)	CO ₂ history	MBC	MBN	EOC	EON	Ni	SR (μg CO ₂ -C g ⁻¹ soil)	SIR
(mg kg ⁻¹ soil)								
<i>Calcarosol</i>								
0-5	aCO ₂	47 _{ab}	13 _b	33 _{ab}	13 _c	18 _c	145 _a	780 _b
	eCO ₂	61 _b	20 _{bc}	52 _b	12 _c	14 _{bc}	265 _b	870 _b
5-10	aCO ₂	11 _a	5 _a	15 _a	5 _a	5 _{ab}	85 _a	525 _a
	eCO ₂	42 _{ab}	6 _a	39 _{ab}	4 _a	3 _a	160 _a	600 _a
<i>Chromosol</i>								
0-5	aCO ₂	148 _c	46 _e	93 _c	48 _e	55 _e	460 _c	1965 _d
	eCO ₂	83 _b	34 _d	111 _c	43 _e	45 _d	490 _c	2005 _d
5-10	aCO ₂	90 _b	22 _c	109 _c	27 _d	14 _{bc}	280 _b	1125 _c
	eCO ₂	91 _b	20 _{bc}	110 _c	26 _d	15 _{bc}	250 _b	1065 _c
<i>Vertosol</i>								
0-5	aCO ₂	176 _d	42 _{de}	48 _b	10 _b	10 _b	205 _b	1155 _c
	eCO ₂	72 _b	40 _{de}	54 _b	6 _a	6 _{ab}	175 _b	1200 _c
5-10	aCO ₂	84 _b	18 _{bc}	27 _{ab}	6 _a	4 _a	160 _{ab}	795 _b
	eCO ₂	43 _{ab}	15 _{bc}	39 _{ab}	4 _a	4 _a	235 _b	780 _b
<i>Significance level</i>								
<i>CO₂</i>		n.s.	n.s.	n.s.	*	n.s.	*	n.s.
<i>Soil</i>		***	***	**	***	***	***	***
<i>Depth</i>		***	***	**	***	***	***	***
<i>CO₂ × Soil</i>		**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<i>CO₂ × Depth</i>		*	n.s.	n.s.	n.s.	**	n.s.	n.s.
<i>Soil × Depth</i>		n.s.	*	*	***	***	***	***
<i>CO₂ × Soil × Depth</i>		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
n.s., *, ** and *** indicate, $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$, respectively.								

n.s., *, ** and *** indicate, $P > 0.05$, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Table 2

Table 2 Functional gene abundance (gene copies g⁻¹ soil) in 0-5 and 5-10 cm layers of Calcarosol, Chromosol and Vertosol soils under ambient CO₂ (aCO₂, 390 ppm) and elevated CO₂ (eCO₂, 550 ppm) for 5 years. For each parameter, means with the same letter are not significantly different at *P*≤0.05.

Soil & depth (cm)	CO ₂ history	Laccase (×10 ⁷)	Cellulase (×10 ⁵)	Alkaline peptidase (×10 ⁷)	Neutral peptidase (×10 ⁴)	Archaeal <i>amoA</i> (×10 ⁷)	Bacterial <i>amoA</i> (×10 ⁵)	Nitrite oxido reductase (×10 ⁵)	Nitrate reductase (×10 ⁸)	Nitrite reductase (×10 ⁸)	Nitrous oxide reductase (×10 ⁸)
<i>Calcarosol</i>											
0-5	aCO ₂	2.2 _b	3.4 _b	0.7 _b	1.2 _{ab}	9.7 _d	8.7 _d	2.8 _b	1.2 _b	1.2 _b	4.5 _b
	eCO ₂	2.9 _{bc}	3.9 _b	0.7 _b	1.7 _b	9.7 _d	7.6 _d	3.0 _b	1.9 _c	2.3 _c	5.4 _b
5-10	aCO ₂	0.6 _a	1.5 _a	0.4 _{ab}	0.5 _a	1.4 _a	0.5 _a	0.5 _{ab}	0.5 _a	0.5 _a	1.3 _a
	eCO ₂	0.9 _a	1.7 _a	0.3 _a	0.6 _a	1.1 _a	0.7 _{ab}	0.5 _{ab}	0.7 _{ab}	0.7 _{ab}	1.5 _a
<i>Chromosol</i>											
0-5	aCO ₂	5.5 _d	15.6 _d	4.1 _e	6.1 _{cd}	9.1 _d	18.5 _e	9.5 _c	9.8 _h	9.8 _d	31.7 _f
	eCO ₂	4.2 _{cd}	19.4 _d	3.5 _e	5.4 _{cd}	5.3 _{bc}	8.3 _d	4.0 _b	7.9 _g	7.9 _d	27.4 _{ef}
5-10	aCO ₂	2.2 _b	8.0 _c	2.5 _{cd}	3.0 _{bc}	2.3 _a	1.1 _{ab}	10.2 _c	5.2 _{ef}	3.4 _c	15.4 _d
	eCO ₂	1.7 _b	5.3 _{bc}	2.0 _c	3.0 _{bc}	1.0 _a	1.6 _b	3.6 _b	3.9 _d	2.7 _c	9.6 _c
<i>Vertosol</i>											
0-5	aCO ₂	15.1 _f	6.9 _c	2.9 _d	8.3 _d	7.7 _{cd}	18.7 _e	2.9 _b	6.2 _f	7.9 _d	27.7 _{ef}
	eCO ₂	11.7 _e	3.9 _b	2.4 _{cd}	4.5 _c	4.3 _b	16.0 _e	1.7 _b	4.8 _{de}	7.7 _d	21.4 _e
5-10	aCO ₂	5.5 _d	1.1 _a	0.7 _b	1.8 _b	1.4 _a	5.4 _c	0.6 _a	1.6 _{bc}	1.6 _{bc}	6.0 _b
	eCO ₂	3.7 _c	0.9 _a	0.6 _b	0.9 _a	1.1 _a	4.7 _c	0.4 _{ab}	1.3 _{bc}	1.5 _{bc}	4.6 _b
<i>Significance level</i>											
	CO ₂	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Soil	***	***	***	***	n.s.	***	***	***	***	***
	Depth	***	***	***	***	***	***	***	***	***	***
	CO ₂ × Soil	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
	CO ₂ × Depth	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Soil × Depth	**	*	***	**	n.s.	n.s.	**	**	**	***
	CO ₂ × Soil × Depth	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s., *, ** and *** indicate, *P*>0.05, *P*<0.05, *P*<0.01 and *P*<0.001, respectively.

Table 3

Table 3 Correlations between functional gene abundance (gene copies ng⁻¹ DNA) and total C (TC), total N (TN), C-to-N ratio (C:N), extractable organic N (EON) and microbial biomass C (MBC) of Calcarosol, Chromosol and Vertosol soils under ambient CO₂ (aCO₂, 390 ppm) and elevated CO₂ (eCO₂, 550 ppm) for 5 years. Values in bold are significantly different at $P \leq 0.05$.

Gene / enzyme	Functional process	aCO ₂					eCO ₂				
		TC	TN	C:N	EON	MBC	TC	TN	C:N	EON	MBC
<i>Calcarosol</i>											
Laccase	Polyphenol degradation	0.693	0.675	0.008	0.711*	0.476	0.362	0.372	-0.157	0.612	-0.041
Cellulase	Cellulose degradation	0.438	0.458	0.257	0.292	0.262	0.289	0.355	-0.728*	0.428	-0.271
Alkaline peptidase	Organic N mineralisation	-0.328	0.048	0.052	0.140	-0.071	0.281	0.287	-0.226	0.522	-0.002
Neutral peptidase	Organic N mineralisation	0.173	-0.085	0.238	0.319	-0.012	0.473	0.458	-0.107	0.556	0.349
Archaeal amoA	Nitrification (NH ₄ ⁺ to NO ₃ ⁻)	0.738*	0.795*	-0.095	0.524	0.452	0.595	0.690	-0.452	0.643	0.333
Bacterial amoA	Nitrification (NH ₄ ⁺ to NO ₃ ⁻)	0.667	0.627	-0.214	0.786*	0.833*	0.917**	0.938**	-0.282	0.945***	0.416
Nitrite oxidoreductase	Nitrification (NO ₂ ⁻ to NO ₃ ⁻)	0.681	0.590	-0.089	0.856**	0.500	0.863**	0.845**	-0.101	0.796*	0.250
Nitrate reductase	Denitrification (NO ₃ ⁻ to NO ₂ ⁻)	0.542	0.627	-0.073	0.531	0.452	0.510	0.504	-0.176	0.655	0.214
Nitrite reductase	Denitrification (NO ₂ ⁻ to NO)	0.502	0.602	0.178	0.463	0.167	0.694*	0.670	-0.063	0.803**	0.304
Nitrous oxide reductase	Denitrification (N ₂ O to N ₂)	0.739*	0.868**	-0.347	0.839**	0.500	0.698*	0.683	-0.154	0.841**	0.490
<i>Chromosol</i>											
Laccase	Polyphenol degradation	0.459	0.445	-0.175	0.494	0.182	0.952**	0.976**	-0.286	0.786*	-0.024
Cellulase	Cellulose degradation	0.804*	0.825*	-0.602	0.426	0.398	0.786*	0.833*	-0.095	0.619	-0.286
Alkaline peptidase	Organic N mineralisation	0.662	0.709*	-0.713*	0.209	0.094	0.762*	0.810*	-0.690	0.548	0.286
Neutral peptidase	Organic N mineralisation	0.176	0.176	-0.145	0.303	0.083	0.524	0.429	0.262	0.452	0.667
Archaeal amoA	Nitrification (NH ₄ ⁺ to NO ₃ ⁻)	0.792*	0.786*	-0.404	0.904**	0.437	0.667*	0.714	-0.286	0.738**	-0.071
Bacterial amoA	Nitrification (NH ₄ ⁺ to NO ₃ ⁻)	0.643	0.548	-0.095	0.786*	0.357	0.762*	0.690*	0.143	0.786**	-0.071
Nitrite oxidoreductase	Nitrification (NO ₂ ⁻ to NO ₃ ⁻)	0.270	0.314	-0.481	-0.247	0.227	-0.548	-0.524	0.786	-0.571	-0.310
Nitrate reductase	Denitrification (NO ₃ ⁻ to NO ₂ ⁻)	0.441	0.444	-0.300	0.470	0.114	0.690*	0.643	-0.476	0.714	0.571
Nitrite reductase	Denitrification (NO ₂ ⁻ to NO)	0.311	0.307	-0.184	0.745*	0.191	0.833**	0.881**	-0.262	0.762**	-0.024
Nitrous oxide reductase	Denitrification (N ₂ O to N ₂)	0.857**	0.833**	-0.293	0.621	0.449	0.976***	0.929***	-0.286	0.857**	0.238
<i>Vertosol</i>											
Laccase	Polyphenol degradation	0.036	-0.497	0.849*	-0.509	0.089	0.316	0.295	-0.006	0.266	0.094
Cellulase	Cellulose degradation	0.645	0.830*	-0.642	0.898*	0.465	0.399	0.428	-0.452	0.263	0.141
Alkaline peptidase	Organic N mineralisation	0.839*	0.469	0.122	0.449	0.708	0.548	0.478	0.124	0.719*	0.408
Neutral peptidase	Organic N mineralisation	0.580	0.515	-0.240	0.549	0.766	0.853**	0.829*	-0.345	0.529	0.781*
Archaeal amoA	Nitrification (NH ₄ ⁺ to NO ₃ ⁻)	0.644	0.257	0.264	0.239	0.662	0.689*	0.648	-0.111	0.593	0.489
Bacterial amoA	Nitrification (NH ₄ ⁺ to NO ₃ ⁻)	0.101	0.280	-0.438	0.374	0.712	0.919**	0.929**	-0.552	0.635	0.829*
Nitrite oxidoreductase	Nitrification (NO ₂ ⁻ to NO ₃ ⁻)	0.934**	0.605	0.017	0.565	0.571	0.634	0.567	0.056	0.575	0.479
Nitrate reductase	Denitrification (NO ₃ ⁻ to NO ₂ ⁻)	0.590	0.071	0.488	0.062	0.583	0.702*	0.657	-0.107	0.601	0.484
Nitrite reductase	Denitrification (NO ₂ ⁻ to NO)	0.588	0.162	0.311	0.185	0.773	0.760*	0.706*	-0.116	0.635	0.626
Nitrous oxide reductase	Denitrification (N ₂ O to N ₂)	0.694	0.540	-0.146	0.550	0.745	0.799*	0.773*	-0.267	0.647	0.603

*, ** and *** indicate, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Figure 1

Fig. 1 Total C (a, b, c), total N (d, e, f) and C:N (g, h, i) in 0-5 and 5-10 cm layers of Calcarosol (left), Chromosol (middle) and Vertosol (right) soils under ambient CO₂ (aCO₂, 390 ppm) and elevated CO₂ (eCO₂, 550 ppm) for 5 years. Error bars indicate standard error of the mean. For each parameter, means with the same letter are not significantly different at $P \leq 0.05$. Letters for total C and N data are for log(x) transformed data. Note the different scales on the Y-axis.

Figure 2

Fig. 2 Bacteria (a) and fungi (b) in the 0-5 cm layer of Calcarosol, Chromosol and Vertosol soils under ambient CO₂ (aCO₂, 390 ppm) and elevated CO₂ (eCO₂, 550 ppm) for 5 years.

Figure 3

Fig. 3 Soil chemical properties (a,c) and functional gene abundance (gene copies ng⁻¹ DNA) (b,d) in the 0-5 cm (a,b) and 5-10 cm (c,d) layers of Calcarosol, Chromosol and Vertosol soils under ambient CO₂ (aCO₂, 390 ppm) and elevated CO₂ (eCO₂, 550 ppm) for 5 years.