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Long-term CO_2 enrichment alters the diversity and function of the microbial community in soils with high organic carbon

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Abstract
The response of soil microorganisms to elevated atmospheric CO_2 (eCO_2) has the potential to alter the regulation of soil biogeochemical processes including carbon and nutrient cycling. A mechanistic understanding of this microbial response in agricultural systems is essential due to the potential impact on soil quality. This study used an eight-year free-air-CO_2 enrichment (SoilFACE) experiment to assess the microbial response to eCO_2 in three major agricultural soils (Chromosol, Vertosol, and Calcarosol) planted annually with grain crops. Elevated CO_2 increased the number of operational taxonomic unit (OTU) by 14.3%, 13.3% and 4.1% and the Shannon diversity by 3.7%, 4.4%, and 2.6% in the top 5-cm soil layer of the Chromosol, Calcarosol and Vertosol, respectively. The relative abundance of the oligotroph *Acidobacteriaceae Subgroup 1* in the top 5-cm soil of the Chromosol and Vertosol was significantly increased by eCO_2. Elevated CO_2 did not affect community diversity in the 5–10 cm soil layer. The functional attribute analysis of the bacterial communities showed that eCO_2 increased pectin and benzene degradation, the pentose phosphate pathway and the production of phytase-6 in the top 5-cm soil of the Chromosol. These results suggest that eCO_2 increases the presence of oligotrophs in the bacterial community and overall mineralization of soil organic carbon (SOC) in surface soils with high SOC. Changes in microbial function due to eCO_2 likely impact the stability of SOC and, consequently, the quality of farming soils for sustainable crop production.
Keywords: 16S gene; priming effect; climate change; microbial function attribute; SOC decomposition

1. Introduction

Due to anthropogenic activities, the atmospheric CO_2 concentration has exceeded pre-industrial levels by 40% (Ciais et al., 2013; Terrer et al., 2018), and has increased at a rate of 1.7 ppm per year over the last 60 years (Kuzyakov et al., 2019). This rapid increase in atmospheric CO_2 is expected to continue for decades and impact global carbon and nutrient cycling through changes in plant-soil-microbe interactions.

Elevated CO_2 (eCO_2) generally stimulates photosynthesis in plant species, leading to increased photosynthetic carbon (C) input into soils (Amthor, 2001; Morgan et al., 2005; Sánchez-Carrillo et al., 2018). However, findings have been inconsistent on the impact of eCO_2 on soil C (West et al., 2006; Butterly et al., 2016), nitrogen (N) (Müller et al., 2009; McKinley et al., 2009; Rütting et al., 2010; Chen et al., 2012), and phosphorus (P) (Jin et al., 2017) of various ecosystems.

The variable responses of soil C and nutrients to eCO_2 are primarily due to feedback responses by the soil microbiome driving C, N, P, sulphur (S) and metal cycles (Bardgett et al., 2008; Zhou et al., 2012), and in turn are affected by characteristics of the soil environment and its management. Previous studies have indicated that eCO_2 can increase (average 67%; Dunbar et al., 2014; Okubo et al., 2015;

Yu et al. 2016) and decrease (average 27%; Dunbar et al., 2014; Yu et al. 2016) individual microbial genera in soils with changes likely to have impacts on soil nutrient cycling and plant productivity. Research has also found, however, that eCO_2 does not significantly change the overall bacterial community level structure under pasture grown on Mollic Psammaquent and Fluvic Gleysol soils (Brenzinger et al., 2017; Xia et al., 2017). These discrepancies in the microbial response indicate that either the temporal and scalar scope of these studies are not sufficient to detect changes to the microbial community or other edaphic factors such as soil type play a role in the effects of eCO_2.

Previous studies on the response of microorganisms to eCO_2 have been limited in scope, using either a short experimental period (five weeks to one year) or conducting in a controlled environment (Peterson et al., 2008; Drake et al., 2016; Yu et al., 2016; Liu et al., 2017). Long-term field-based studies across a range of soils are required to confirm the influence of eCO_2 on soil microbial community dynamics, especially regarding selection of microorganisms with different life strategies (Xu et al., 2008; Lian et al., 2017). Copitorophic and oligothrophic microorganisms vary in their ability to consume labile nutrients, compete for nutrient-poor substrates, and differ in growth rates (Fierer et al., 2007). Long-term eCO_2 has been suggested to favour the growth of oligothrophs rather than copitorophs due to the decrease of labile nutrients in soils (Jin et al., 2019). The responses of copiotrophs and oligotrophs to eCO_2 are likely to have implications on nutrient availability in soils, which in turn would impact plant growth and inevitably affect the soil C stock and quality in the future (Huang et al., 2015; Terrer et al., 2018). For example, Butterly et al. (2016) observed that five years of eCO_2 significantly altered specific C/N transformation-associated genes in a free air CO_2 enrichment (FACE) experiment covering various soils through quantitative PCR. Therefore, an in-depth study is warranted on the effect of eCO_2 on the phylogeny and functional attributes of soil microbial communities using high-throughput sequencing and emerging strategies for predictive functional profiling. Such a study would provide insights into the potential impact of copiotroph and oligotroph dynamics on nutrient cycling under long-term eCO_2.

This study used an eight-year FACE to compare the structure of bacterial communities at the genus level and their functions on C and nutrient cycles in response to eCO_2 across three major farming soils. We hypothesized that long-term eCO_2 would benefit the growth of oligotrophs rather than copiotrophs due to the decrease of labile nutrients in soil, and that this increase in oligotrophs would be stronger in soils with high SOC, providing more substrates for SOC mineralization under eCO_2.

2. Materials and methods

2.1. Experimental design

A soil free air CO_2 enrichment (SoilFACE) experiment was established in 2009 at the Agriculture Victoria Research station, Horsham, Victoria, Australia (36°44'57"S, 142°06'50"E). The experimental site was characterised by a Mediterranean climate. During the experimental period, the annual rainfall ranged from 295–630 mm, maximum mean temperatures from 21.4 to 23.1 ºC, and minimum mean temperatures from 7.2 to 8.1 ºC. This experiment consisted of two CO_2 treatments, i.e. eCO_2 (550 ppm) and ambient CO_2 (aCO_2) (390 ppm) and three soils. A randomised split-plot design was used with CO_2 as the main plot, and soil type as the subplot nested within the CO_2 plot (Butterly et al. 2016). The subplots (soils) were randomly allocated in each main plot (Fig. S1). There were four bunkers (as four replicates) for each CO_2 treatment. Each bunker was enclosed with stainless steel tubes to form an octagon (side length: 1.86 m). The CO_2 was supplied through these tubes to maintain the target concentration in the bunker with an infrared gas analyser installed in the centre of the bunker to facilitate the control system operating the gas supply. The FACE system used to achieve eCO_2 was further detailed in Mollah et al. (2011).

The three soils were Chromosol (Luvisol), Vertosol (Vertisol) and Calcarosol (Calcic Xerosol) (Isbell 1996; FAO-UNESCO 1976). These are major soil types in dryland cropping systems of South-Eastern Australia. The general physicochemical characteristics of the three soils are summarized in Table 1. The intact soil mesocosms (30-cm diameter; 100-cm depth) in bunkers were arranged as previously outlined (Jin et al. 2017). Briefly, four mesocosms for each soil were randomly arranged in each bunker, resulting in total 12 mesocosms being placed into the ground of each bunker. The soil surface of each mesocosm was aligned with the surface of the surrounding paddock. The layout of the experimental design in the field is shown in Figure S1.

Crops were grown following a rotation for all soils, which included field pea (*Pisum sativum* L. cv. PBA Twilight), wheat (*Triticum aestivum* L. cv. Yitpi), field pea, wheat, canola (*Brassica napus* L. cv. Hyola 50), wheat, canola and wheat in 2009, 2010, 2011, 2012, 2013, 2014, 2015 and 2016, respectively (Jin et al. 2019). Triple superphosphate was applied with the seed at sowing each year at the rate of 15 kg P ha^{-1}. Fifty kg N ha^{-1} in urea was surface-applied for wheat and canola only. Other nutrients were at adequate levels and hence were not applied. At maturity, plant shoots were harvested at the base of the stems. After removal of grains, the shoot residues were chopped into < 2 cm segments and returned to their respective mesocosms.

2.2. Soil sampling and measurements

In December 2016 following crop harvest, soils were sampled at two depths: 0–5 cm and 5–10 cm. For each soil type, eight cores (15.7 cm^3 per core) were taken from the four mesocosms (two cores per mesocosm) in each bunker and combined to form a composite sample as a replicate. Then, each soil sample was split into three parts: one part (approximately 2 g) was stored at -80 ºC for DNA extraction and sequencing. The second part (approximately 30 g) was kept at 4 °C for measurements of ammonium (NH_4^+), nitrate (NO_3^-) and dissolved organic C (DOC). The remaining portion (approximately 30 g) was air-dried for chemical measurements including total soil C (SOC) and N, Olsen phosphorus (P), available potassium (K), and available sulphur (S) and pH. The SOC and total N in soil were determined using a CHNS elemental analyser (Perkin Elmer 2400 Series II, USA) after the soil was finely ground and homogenised using a ball mill (Retsch MM400, Hanau, Germany). The pH was determined using a Wettler Toledo 320 pH meter after shaking the soil in water (1:5 = w:v) for 30 min. A continuous flow analyser (SKALAR SAN^{++}, Skalar, the Netherlands) was used to determine NH_4^+ and NO_3^- after extraction in 2 M KCl for 1 h (Miranda et al., 2001). Colorimetrical methods of indophenol blue (Kempers and Kok 1989) and azo dye (Greiss reaction) (Brewer and Riley 1965) were deployed in this system for determinations of NH_4^+ and NO_3^-, respectively. The DOC was determined according to Domanski et al. (2001). Olsen P concentration was measured according to Olsen et al. (1954).

2.3. DNA extraction, 16S rRNA gene amplification, and sequencing

Soil DNA was extracted with a Fast DNASPIN Kit for Soil (Qbiogene Inc., Carlsbad, CA, USA). The extracts were then dissolved in 100 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and then was quantified using a NanoDrop spectrophotometer (Bio-Rad Laboratories Inc., Hercules, USA). Regarding MiSeq sequencing, bacterial 16S rRNA genes were amplified with primer 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 909R (5'-CCCCGYCAATTCMTTTRAGT-3') with 12 nt unique barcodes. The cycling conditions of the PCR were an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 40 s, 56 °C for 60 s, and 72 °C for 60 s, and a final extension at 72 °C for 10 min. The PCR products were then sequenced on an Illumina MiSeq platform (Caporaso et al., 2011, 2012).

After sequencing was undertaken, the raw sequences were sorted based on the indices, and trimmed for sequence quality using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (version 1.17; http://qiime.org/). One operational taxonomic unit (OTU) was defined as similarities of > 97% for sequences. In total, there were 1,211,745 high-quality and chimera-free reads with an average length of 397 bp obtained. As the sequence number varied from 17,960 to 32,125 among samples, a subset of 17,960 reads was randomly selected for each sample before further analysis. Ribosomal Database Project (RDP) database was used to identify phylotypes. Raw fastq files for this project have been deposited with the NCBI SRA database and can be accessed using Bioproject ID: PRJNA521425.

2.4. Statistical analysis of functional traits

In accordance with the BugBase guidelines, taxonomic assignments were done using closed reference OTU picking against the Greengenes gg 97 reference set (https://www.drive5.com/usearch/manual/download_gg97.html). Briefly, raw, demultiplexed, fastq files were re-barcoded, joined and quality-filtered using UPARSE OTU clustering pipeline (Edgar 2013). Merged reads were discarded if the merged region contained > 10 bp different or < 80% similarity. Reads with total expected errors > 1.0 or a merged length < 200 bp were discarded as well. Operational taxonomic units (OTUs) were clustered with a 97% similarity cut-off using USEARCH

closed reference OTU picking script (USEARCH version 10.0.240 http://drive5.com/uparse/) against the Greengenes gg 97 reference set.

Basic functional traits were analysed using BugBase (Ward, Larson et al., 2017). In addition to default analyses, which incorporate stress tolerance, biofilm formation, pathogenicity, Gram positive/negative and anaerobic/aerobic traits, custom BugBase files were run to test for genes related to N cycling, S cycling, DNA repair, methanogenesis and methanotrophy, and plant residue consumption. Approximately 37% of OTUs (1866 of 5068) from the input table matched the 203452 available OTUs database of phylogenetic investigation of microbial communities by reconstruction of unobserved states (PICRUSt).

Validation of genes to be included within each custom file relied on existing literature and expert knowledge. The KEGG database contains compiled information on functional pathways and their contributing genes. As such, where this information was available for a given function (e.g. pentose phosphate pathway), gene sets used for custom BugBase analyses were derived from the KEGG orthologs (Kos) present in relevant KEGG modules. With respect to plant residue degradation, for which there is no specific KEGG module, we investigated degradation pathways for lignin, which is a main structural polymer of plants, as well as benzoate which is a breakdown product of lignin (Parales and Harwood, 2002). As the phytase genes were of direct interest, the prevalence of phytase -3 (K01083) and phytase-6 (K01093) were examined directly.

2.5. Statistical analyses

UniFrac statistical analysis was performed online at http://bmf.colorado.edu/unifrac/ to provide the index of distances based on community phylogenetic structure between each pair of samples (Lozupone and Knight, 2005; Hamady and Knight, 2009). Principal coordinates analysis (PCoA) was then used to demonstrate patterns of similarity (Weighed UniFrac) in microbial community structure between treatments, i.e. β diversity (Li et al., 2014). The PCoA was performed using the program R version 3.1.2 for Windows (R Development Core Team 2010). The α diversity parameters, including Ace, Chao estimator, Shannon's diversity index and Simpson index were estimated with the MOTHUR program (http://www.mothur.org) (Chelius & Triplett 2001). Ace and Chao are considered as community richness. Shannon and Simpson indices represent community diversity and evenness, respectively.

An analysis of variance (ANOVA) (Steel and Torrie, 1980) was performed with a linear-mixed model fit by residual maximum likelihood in Genstat 13 (VSN International, Hemel Hemspstead, UK). With this model, we estimated the effect of eCO_2 on soil pH, concentrations of SOC and total N, NO_3^-, NH_4^+, DOC, Olsen P, available K and available S, and the relative abundance of the bacterial groups at the genus and OTU levels. Only genera that were significantly affected by treatment ($P < 0.05$), were presented. This significant level was estimated according to the least significant difference (LSD). The correlations of α diversity parameters of bacterial community and genus abundance with soil chemical properties were performed at the significant level of $P < 0.05$, $P < 0.01$ and $P < 0.001$. Thirty of top abundant genera in the community were analysed for correlations with soil chemical properties, which were shown in a heatmap. Differences in the predicted abundances of functional genes were assessed in R using permutation ANOVAs via the 'aovp' function from the package 'lmperm'.

3. Results

Sequencing depth across treatments was sufficient to significantly represent the bacterial community diversity of the soil communities with rarefaction coverage ranging from 0.947 to 0.988. Elevated CO_2 significantly increased the OTU number, Ace and Chao estimator values of the top 5 cm of all three soils, while there was no significant CO_2 effect seen in the 5–10 cm soil layer. Similarly, compared to aCO_2, eCO_2 increased Shannon index by 3.7%, 4.6% and 2.6% in the top 5-cm layer for the Chromosol, Calcarosol and Vertosol, respectively. Simpson index decreased significantly under eCO_2 and the extent of this decrease was greater in the Calcarosol than in the Chromosol and Vertosol (Table 2). However, eCO_2 did not alter Shannon and Simpson indices in the 5–10 cm layer of any soil. Elevated CO_2 decreased concentrations of SOC, DOC and total N in the top 5 cm of the Chromosol ($P < 0.05$). Elevated CO_2 also lowered the concentrations of NO_3^-, NH_4^+, Olsen P, available K and available S irrespective of soil type and soil depth ($P < 0.05$ to < 0.01), but did not alter soil pH significantly ($P > 0.05$) (Table S1). The OTU number, Ace, Shannon index and Chao were correlated negatively with concentrations of SOC, total N, NO_3^- and NH_4^+ but positively with available K concentration and pH

(P <0.05 to <0.001); the exception was for Ace and Chao against NO_3^- concentration (P >0.05) (Table 3). In contrast, Simpson index was correlated positively with concentrations of NO_3^- and NH_4^+ but negatively with available K concentration and pH (P <0.05 to <0.001).

Bacterial communities differed significantly among three soils as indicated by PCoA (Fig. 1). However, eCO_2 did not alter the structure of overall bacterial communities in either soil layer or any soil type when analysed by ANOSIM (Clarke, 1993) and ADONIS (Anderson, 2001) (P >0.05) (Table S2).

Although eCO_2 did not generally impact on the bacterial community structure, several genera had a significant response. The extent of this response varied between soil types in the top 5 cm (Fig. 2). The abundance of *Acidobacteriaceae_Subgroup 1* increased from 2.1% to 2.9%, and from 0.03% to 0.5% in the 0–5 cm layer of the Chromosol and Vertosol, respectively. Elevated CO_2 significantly decreased the abundance of *norank_c_Acidobacteria* in the Verstisol, but not in the other soils. Similarly, eCO_2 decreased *Skermanella* abundance from 4.0% to 2.9% in the Verstisol, but not in either the Chromosol or the Calcarosol. Elevated CO_2 decreased the abundance of *norank_f_Elev-16S-1332* in the Chromosol, increased it in the Vertosol and did not affect it in the Calcarosol. There was no significant CO_2 effect (P > 0.05) on these genera in the 5–10 cm soil layer (data not shown).

Several genera had close associations with soil chemical characteristics (Fig. 3). Most of 30 genera with top abundance in the microbial community were correlated with SOC, N, available K and pH. For example, the *Acidobacteriaceae_Subgroup 1* was negatively correlated with Olsen P, available K and S, and pH. Moreover, *norank_f_Elev-16S-1332* was correlated positively with concentrations of SOC, total N, NH_4^+ and NO_3^- and negatively with available K and S, and pH. *Skermanella* and *norank_c_Acidobacteria* correlated positively with available K and S, and pH, and negatively correlated with the concentrations of SOC, total N, NH_4^+ and NO_3^-.

Elevated CO_2 and soil type significantly affected the proportion of microorganisms capable of metabolising SOC via the pentose phosphate pathway (Fig. 4A: permutational ANOVA $eCO_{2\,(1,18)}$, P = 0.0068; Soil type $_{(2,18)}$, P< 0.001). This effect was due to an increase in community members capable of the oxidative phase of the pentose phosphate pathway, on which main effects of CO_2 and soil type were significant (Fig. 4B: permutational ANOVA: $eCO_{2\,(1,18)}$, P = 0.0036; Soil type $_{(2,18)}$, P < 0.001). Elevated CO_2 did not affect the proportion of community members capable of producing the non-oxidative phase of the pentose phosphate pathway. The effect of soil type on this microbial function was significant (Fig. 4C: permutational ANOVA: Soil type $_{(2,18)}$, P = 0.045).

The proportion of microorganisms predicted to participate in the degradation of pectin and benzene was significantly affected by eCO_2 and soil type, and their interactions (P < 0.05). Elevated CO_2 only increased the predicted abundance of pectin and benzene degrading microorganisms in the Chromosol (Fig. 5A and C). Irrespective of soil type, eCO_2 increased the proportion of the microorganisms capable of expressing the sucrose specific II component of the PTS system (Fig. 5B: permutational ANOVA: $eCO_{2\,(1,18)}$, P = 0.04)

Whilst the proportion of community members able to produce phytase-3 was affected by soil type, there was a significant interaction between eCO_2 and soil type on the community capacity to produce phytase-6 (Fig. 6). Elevated CO_2 only increased the predicted abundance of phytase-6 producing microorganisms in the Chromosol.

4. Discussion

Increases in the diversity and richness of bacterial communities following long-term eCO_2 exposure under field conditions in this cropping system corresponded to the decrease in SOC content in Chromosol (Table S1). The negative correlations between SOC and Chao, Ace and Shannon indices observed in this study (Table 3) suggests that changes in the diversity of the bacterial community may contribute to SOC degradation in response to eCO_2. In addition to this observed decrease in SOC of the Chromosol, the presence of eCO_2 also decreased the concentrations of soil N and P. The enhanced plant nutrient uptake and SOC decomposition under long-term eCO_2 contributed to this decrease of nutrients in soil (Jin et al., 2017; Xu et al., 2019). This change in SOC and nutrient availability is likely to change the competitive dynamics of the community under long-term eCO_2, selecting against formally abundant copiotrophs in favour of rare oligotrophs.

Under eCO_2, bacterial communities were more diverse as indicated by greater OTU number and Shannon index in soils, with low nutrient levels unfavourable for copiotrophs in comparison to aCO_2

(Table 2). A large-scale study of Mollisols in northeast China, showed decreases in SOC, together with total P, and available N and K, favoured the increase of the bacterial diversity, especially for oligotrophic-associated sub phyla of Deltaproteobacteria (Liu et al., 2014; Ho et al., 2017). The ability of oligothrophs to access recalcitrant C appears to enhance their competitive advantage under long-term exposure of eCO_2.

Despite increased rhizodeposition of C compounds into the soil under eCO_2 (Jin et al. 2014), SOC decreased in the surface soil of the Chromosol (Table S1). The reduction in SOC might result from faster SOC mineralization under eCO_2. This priming effect demonstrated the upregulation of functional attributes related to SOC mineralization (i.e. benzene and pectin degradation; Fig. 5). Xu et al. (2018) also found that eCO_2 significantly increased the priming effect in the rhizosphere of wheat, supporting the decomposition of organic compounds in soils being stimulated under eCO_2.

Long-term CO_2 enrichment increased the proportion of the soil microbial community capable of oxidation of the pentose phosphate pathway and producing phytase-6 in the Chromosol (Figs 4 and 6), likely related to SOC priming. Phytate accounts for 50-60% of organic P in soils (McLaren and Cameron, 1996) and would be an important source of P for soil bacteria to mineralize. In another study using this same FACE system, long-term CO_2 enrichment markedly decreased the concentration of organic P in the Chromosol (Jin et al., 2017), further supporting the findings of this study. The reason for the eCO_2-induced change in the microbial production of phytase-6 in the Chromosol only is likely to be due to the greater proportion of organic P in this high-SOC soil compared to the other two soils (Jin et al., 2017). The Chromosol could provide sufficient substrates of organic P to trigger a microbial response in which alternate P-accessing strategies were up-regulated when P became the limiting factor for microbial growth with the increase of plant-C supply under eCO_2. However, as organic P was already limiting in the other two soils, the increase in C input under eCO_2 would be unlikely to trigger a P-starvation response.

Although the overall bacterial community structures in the three soils were not influenced by eCO_2 (Fig. 1), several individual genera significantly responded to eCO_2. We observed declines in the copiotrophs *Skermanella* belonging to Alphaproteobacteria and *norank_c_Acidobacteria* in the Vertosol, and *norank_f_Elev-16S-1332* belonging to Actinobacteria in the Chromosol (Fig. 2). This decrease correlated with increases in rare oligotrophic microbes such as *Acidobacteriaceae_Subgroup_1*, likely due to their ability to access recalcitrant C components in soils and thus accelerate SOC mineralization. In a previous study, with 71 soil samples collected from a wide range of ecosystems in North America, Fierer et al. (2007) found that oligotrophs played a role in SOC mineralization after copiotrophs are suppressed. In addition, although the direct positive effect of eCO_2 on the abundance of autotrophic genera has been observed in the marine systems, no changes in autotrophic genera were determined in this study. This finding is supported by Kuzyakov et al. (2019), who stated that eCO_2 does not benefit this community in the terrestrial systems.

Interestingly, *norank_f_Acidobacteriaceae_subgroup_1* in the Chromosol and the Vertosol was enriched under eCO_2 (Fig. 2), and the abundance of this genus was negatively correlated with Olsen P (Fig. 3), suggesting that it can adapt to the low-P environments. The Acidobacteria phylum is normally enriched in soils with low nutrient availability and some members in the family of Acidobacteriaceae, such as *Edaphobacter lichenicola*, are able to produce both alkaline and acid phosphatases, naphthol-AS-BI-phosphohydrolase, and galactosidase (Fierer et al., 2007; Belova et al., 2018). However, the genus found in this study has not been identified and there were no microbial attributes to the production of phosphatase observed under eCO_2. Whether this genus is associated with the other processes of organic P mineralization, such as the transformation of phytate in specific soils warrants further investigation.

5. Conclusion

Soil C stability and nutrient cycling in response to eCO_2 environments would be fundamentally attributed to the alternation of soil microbial community composition and functions. This study examined the long-term impacts of eCO_2 on the structure of bacterial communities at the genus level and their functions on C and nutrient cycles in three cropping soils. It showed that eCO_2 stimulated the growth of soil oligotrophs, which likely accelerates the decomposition of SOC in cropping soils, especially in soils with high SOC. The eCO_2-induced decreases of labile nutrients in soil due to

increased nutrient removal by crops may naturally trigger the growth of oligotrophs to mine non-labile nutrients from SOC mineralization. The enrichment of oligotrophic community could indicate the necessity of taking countermeasures to mitigate the negative impact of eCO_2 on the SOC loss. Considering when and where measures need to be counted under the global climate change, it is worth clarifying the threshold of this shift in SOC degradation in response to gradual increases in CO_2 concentration, and which oligotrophic species would dominate the mineralization of SOC compounds under such environments.

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Declarations of interest
The authors declare no conflict of interest.

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Figure legends

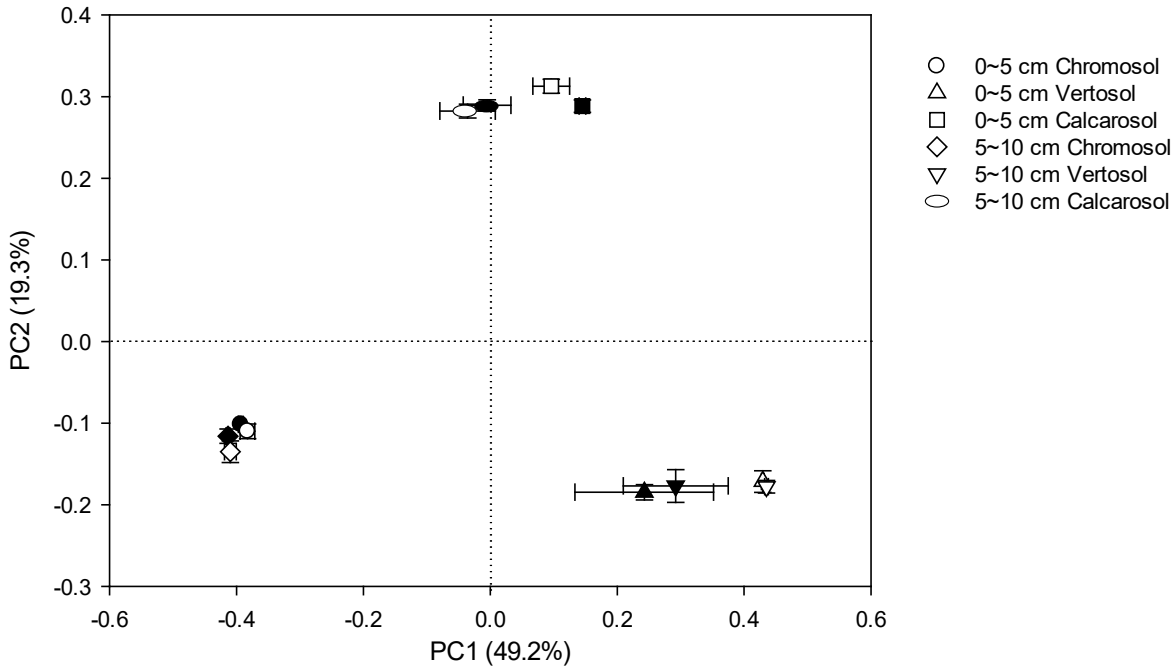

Fig. 1. Principal coordinate analysis (PCoA) of bacterial communities in the depths of 0–5 cm and 5–10 cm in the Chromosol, Vertosol and Calcarosol, where crops were exposed to either aCO_2 (390 ppm) (open symbols) or eCO_2 (550 ppm) (closed symbols) from 2009 to 2015. Bars represent ± standard error (n = 4).

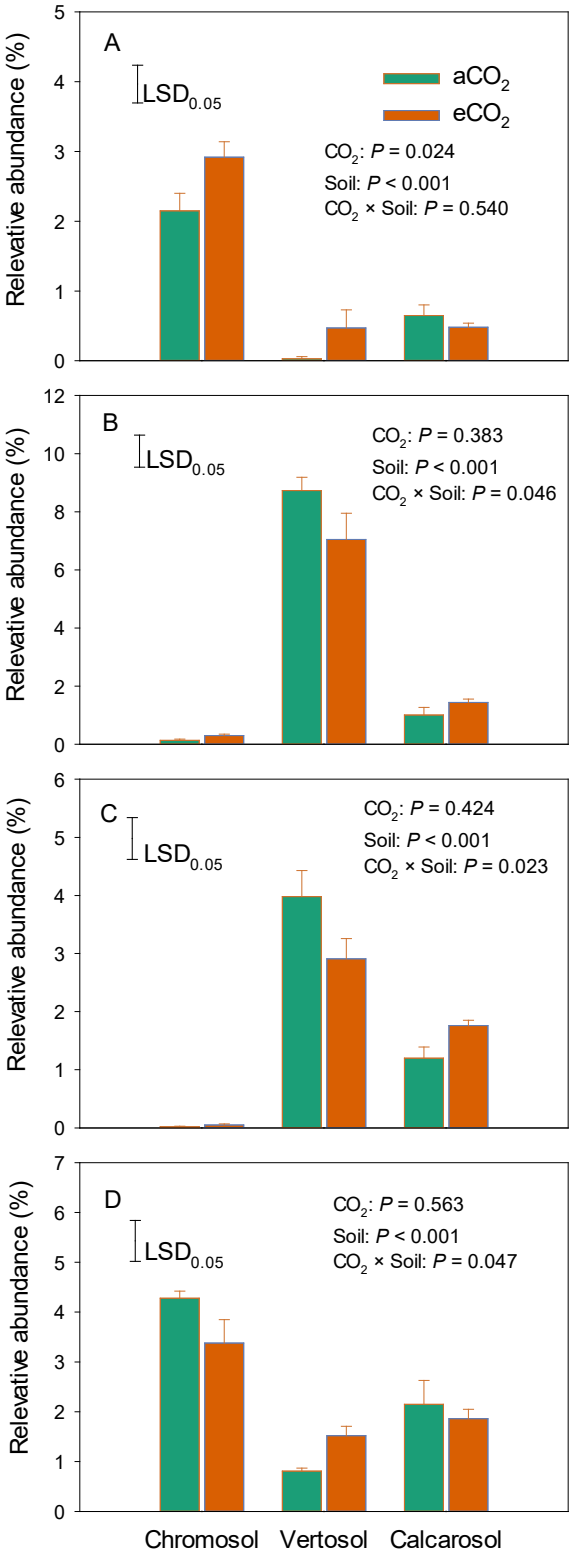

Fig. 2. Elevated CO_2-induced significant changes ($P < 0.05$) in abundances of genera *Acidobacteriaceae_Subgroup_1* (A), *norank_c_Acidobacteria* (B), *Skermanella* (C) and *norank_f_Elev-16S-1332* (D) in the depth of 0–5 cm in the Chromosol, Vertosol and Calcarosol, on which crops had been exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015. Error bars represent the standard error (n = 4). LSD bars ($P = 0.05$) are also shown.

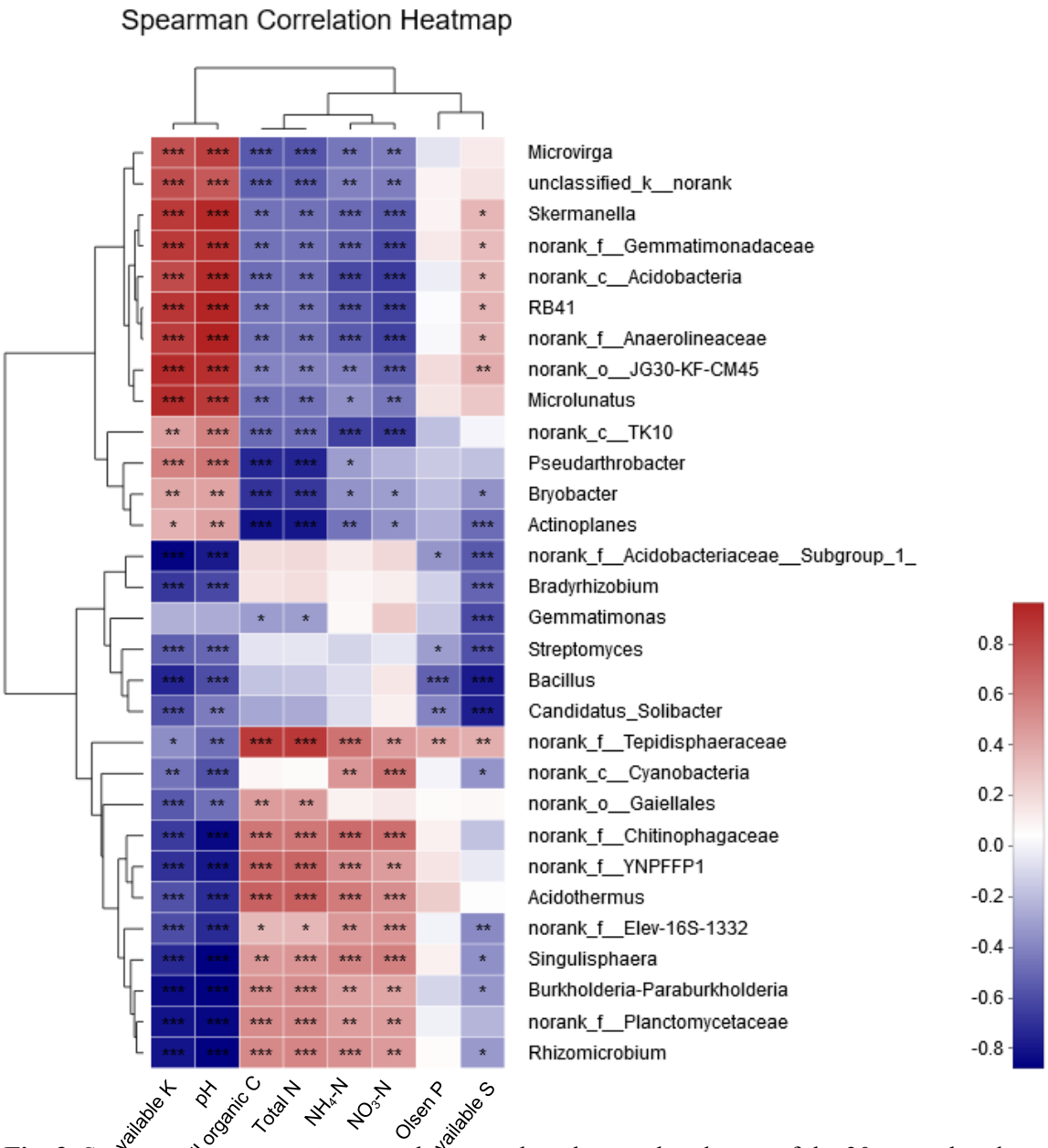

Fig. 3. S[illegible] between the relevant abundances of the 30 most abundant bacteri[illegible] genera (a[illegible] depths, [illegible] types, and CO_2 treatments) and soil chemical properties in the depths of 0–5 cm and 5–10 cm in the Chromosol, Vertosol and Calcarosol, on which crops had been exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015. The colour intensity in each cell indicates the *r* value of the correlation irrespective of soil depth, soil type and CO_2 treatments. Green colour indicates negative correlation and red indicates positive correlation. Asterisks denote significantly correlated genera (*, $P < 0.05$; **, $P< 0.01$ and ***, $P <0.001$).

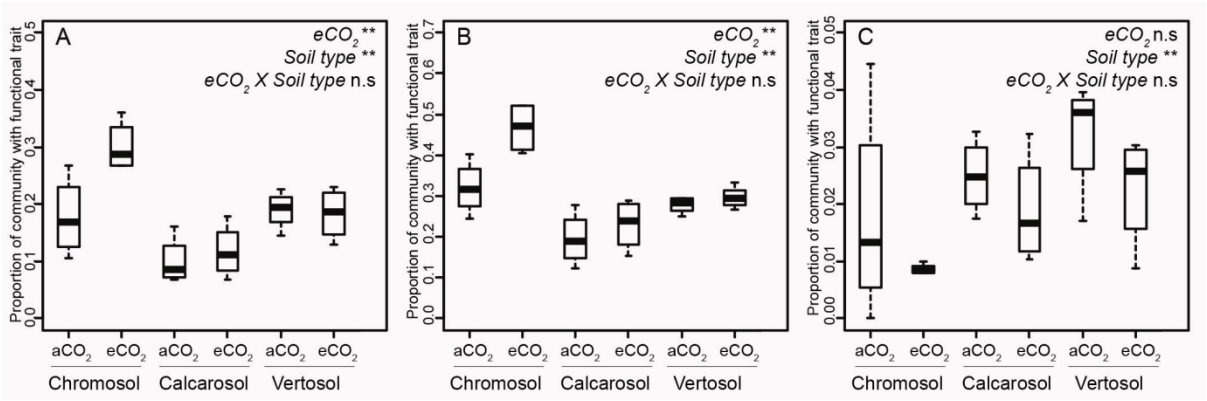

Fig. 4. Box plots of functional attributes associated with the full pentose phosphate pathway (A), the oxidative phase of the pentose phosphate pathway (B) and the non-oxidative phase of the pentose phosphate pathway (C). **, $P < 0.01$; n.s, not significant at $P < 0.05$. Whiskers extend to a maximum of 1.5 × IQR beyond the box (n = 4).

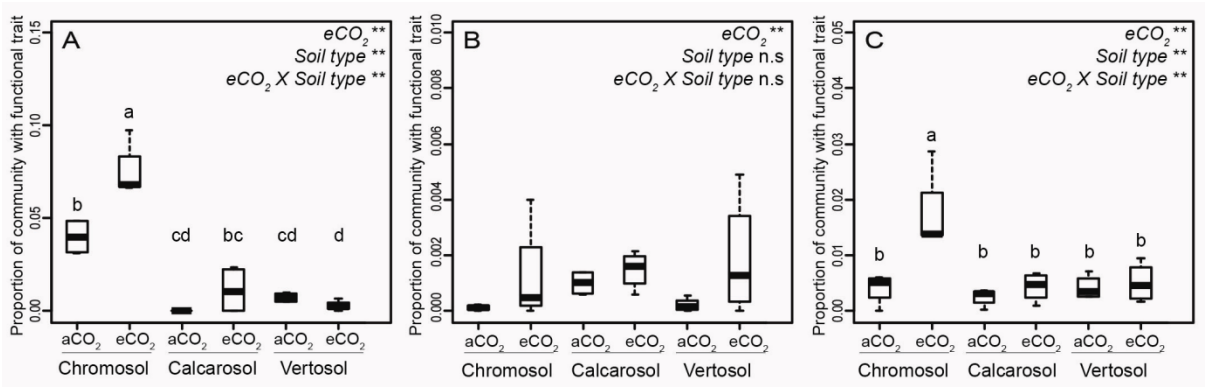

Fig. 5. Box plots of functional attributes associated with pectin degradation (A), the sucrose specific II component of the PTS system (B) and benzene degradation (C). **, $P < 0.01$; n.s, not significant at $P \leq 0.05$. Whiskers extend to a maximum of 1.5 × IQR beyond the box (n = 4). Values with a same letter are not significantly different between treatments ($P = 0.05$).

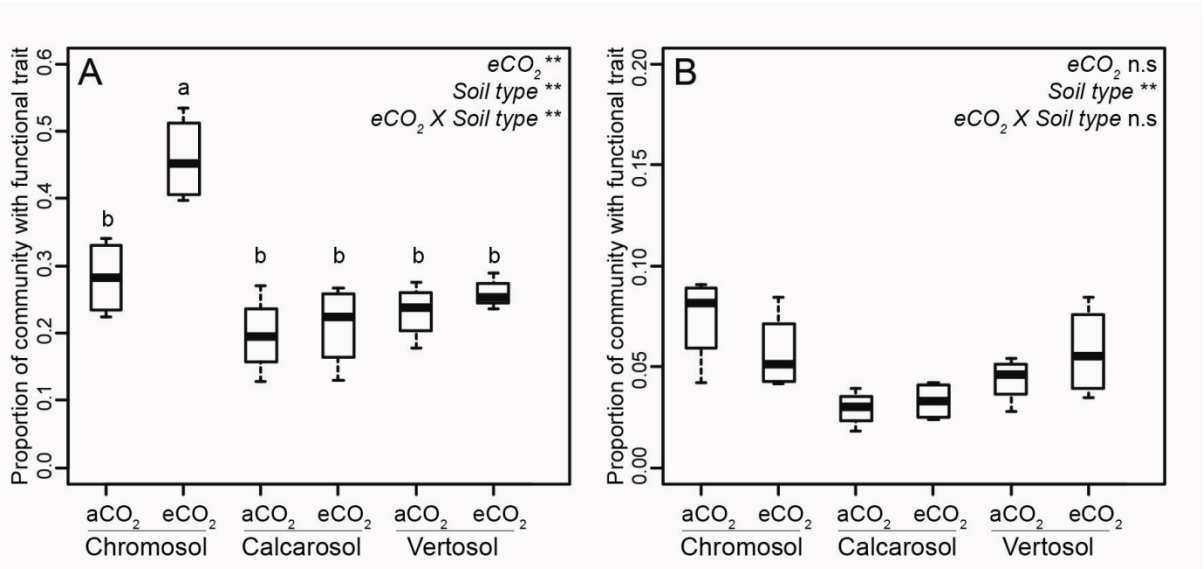

Fig. 6. Box plots of functional attributes associated with the production of phytase-6 (A), and phytase-3 (B). **, $P < 0.01$; n.s, not significant at $P < 0.05$. Whiskers extend to a maximum of 1.5 × IQR beyond the box (n = 4). Values with a same letter are not significantly different between treatments ($P = 0.05$).

Table 1. Concentrations of organic carbon (SOC), total N, pH, and sand, silt and clay contents in the top 10-cm soil of the Chromosol, Vertosol and Calcarosol soils before the experiment commenced in 2009

Soil	SOC	Total N	pH	Sand	Silt	Clay
	(g kg^{-1} soil)			(%)		
Chromosol	46.6	3.9	4.5	16	66	18
Vertosol	11.0	0.8	7.7	12	37	51
Calcarosol	4.4	0.4	5.9	84	10	6

Table 2. Summary of rarefaction coverage, number of OTUs, Ace, Chao, Shannon index, and Simpson index for bacterial communities in the depths of 0–5 cm and 5–10 cm of the Chromosol, Vertosol and Calcarosol, on which crops had been exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015. Values are means of four replicates. Parameters are followed with LSD values correspond to the CO_2 effect at $P = 0.05$, and significant levels of main effects of CO_2 and soil and their interactions (two-way ANOVA). The asterisk after the LSD value denotes that the LSD is for the CO_2 effect × soil interaction.

Soil	Chromosol		Vertosol		Calcarosol		LSD ($P = 0.05$)	CO_2	Soil	CO_2× Soil
	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2				
0-5 cm										
Coverage	0.988	0.985	0.978	0.974	0.974	0.977	-	-	-	-
OTU number	1247	1425	2054	2138	1725	1955	69	0.001	<0.001	0.506
Ace	1538	1777	2600	2769	2252	2472	114	0.004	<0.001	0.906
Chao	1555	1816	2596	2735	2284	2483	132	0.010	<0.001	0.688
Shannon index	5.68	5.89	6.21	6.37	5.89	6.15	0.07	<0.001	<0.001	0.568
Simpson index	0.010	0.008	0.006	0.004	0.014	0.008	0.002*	0.013	<0.001	0.044
5-10 cm										
Coverage	0.987	0.983	0.976	0.974	0.976	0.978	-	-	-	-
OTU number	1245	1370	2118	2124	1957	2026	227	0.498	<0.001	0.733
Ace	1550	1747	2642	2767	2453	2518	278	0.298	<0.001	0.812
Chao	1548	1761	2635	2719	2497	2525	276	0.373	<0.001	0.633
Shannon index	5.64	5.71	6.26	6.33	6.30	6.27	0.25	0.742	<0.001	0.751
Simpson index	0.009	0.009	0.006	0.005	0.006	0.006	0.002	0.954	<0.001	0.703

-, not applicable.

Table 3. The linear correlations between α diversity of bacterial community and soil chemical properties in the depths of 0–5 cm and 5–10 cm of the Chromosol, Vertosol and Calcarosol, on which crops had been exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2016. Asterisks denote significant correlations (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

	Soil organic C	Total N	NO_3^-	NH_4^+	Olsen P	Available K	Available S	pH
OTU number	-0.894***	-0.895***	-0.587*	-0.807**	-0.109	0.687*	0.039	0.902***
Ace	-0.989***	-0.894***	-0.559	-0.794*	0.118	0.696*	0.030	0.906***
Chao	-0.908***	-0.909***	-0.542	-0.802**	0.126	0.673*	0.050	0.896***
Shannon index	-0.831***	-0.834***	-0.645*	-0.794**	0.072	0.624*	0.008	0.823**
Simpson index	0.359	0.355	0.846***	0.602*	-0.148	-0.624*	-0.343	-0.622*

Supplementary Information

Long-term CO_2 enrichment distinctively alters the diversity and functions of microbial communities in three contrasting soils

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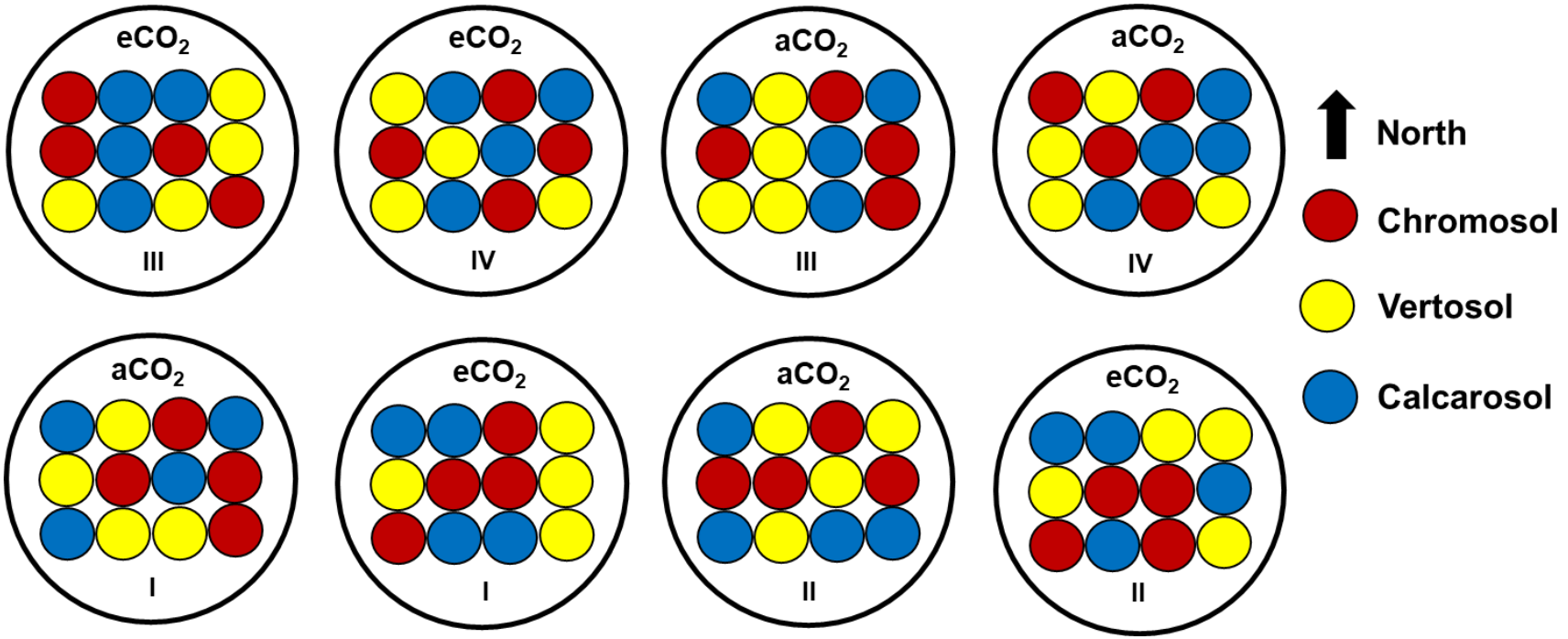

Fig. S1. The graphic diagram showing the experimental design. Each CO_2 treatment had four FACE bunkers, and three soils, Chromosol, Vertosol and Calcarosol, with each having four mesocosms were randomly placed in each bunker. Crops were grown under either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015. I, II, III and IV indicate respective field replicates.

Table S1. Selected chemical properties of the soils from the depths of 0–5 cm and 5–10 cm of the Chromosol, Vertosol and Calcarosol, on which crops had been exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015. Values are means of four replicates ± standard error. Parameters are followed with LSD values correspond to the CO_2 effect at $P = 0.05$, and significant levels of main effects of CO_2 and soil depth and their interactions (two-way ANOVA). The asterisk denotes that the LSD is for the CO_2 effect × soil interaction.

Soil & depth (cm)	CO_2	SOC (mg g^{-1} soil)	Total N	DOC (μg g^{-1} soil)	NO_3^--N	NH_4^+-N	Olsen P	Available K	Available S	pH
Chromosol										
0–5	aCO_2	43.9±0.7	3.44±0.04	302±37	2.26±0.16	3.80±0.62	13.8±0.33	65.4±7.7	14.8±2.1	5.03±0.06
	eCO_2	38.5±0.9	3.08±0.11	226±9	2.17±0.30	2.57±0.14	12.1±0.63	55.1±4.0	14.7±2.0	5.14±0.02
5–10	aCO_2	40.4±0.1	3.25±0.04	197±10	1.51±0.04	2.54±0.29	8.2±0.54	44.7±7.2	13.1±1.4	5.00±0.02
	eCO_2	36.8±1.3	2.96±0.08	184±9	1.34±0.11	1.83±0.05	6.3±0.54	40.4±3.6	10.8±0.7	5.13±0.04
Vertosol										
0–5	aCO_2	10.4±0.3	0.93±0.05	98±1	1.65±0.22	1.70±0.12	14.4±2.32	479.0±8.9	24.1±3.3	7.67±0.17
	eCO_2	10.7±0.8	0.88±0.13	98±10	1.00±0.09	1.71±0.09	11.7±0.45	391.0±31.2	15.5±1.1	7.38±0.28
5–10	aCO_2	10.2±0.1	0.92±0.06	92±8	0.90±0.07	1.20±0.09	10.2±0.44	437.4±10.4	24.8±5.3	7.92±0.10
	eCO_2	9.5±0.1	0.83±0.04	98±9	0.92±0.04	1.32±0.08	7.4±0.28	353.2±43.4	12.9±0.1	7.23±0.41
Calcarosol										
0–5	aCO_2	4.3±0.8	0.39±0.04	74±3	2.82±0.76	2.18±0.17	7.1±0.29	94.4±8.5	3.0±0.2	6.23±0.14
	eCO_2	4.2±0.4	0.36±0.08	62±2	1.28±0.10	1.61±0.16	6.2±0.26	90.0±5.0	3.3±0.1	6.38±0.05
5–10	aCO_2	3.2±0.8	0.31±0.07	58±4	1.05±0.09	1.05±0.07	7.0±0.92	66.1±6.4	1.8±0.1	6.11±0.14
	eCO_2	3.2±0.5	0.29±0.04	55±7	0.81±0.05	0.99±0.06	5.7±0.17	59.0±3.6	1.6±0.2	6.10±0.02
Significance at 0-5 cm										
LSD ($P = 0.05$)		0.99*	0.15*	24.4	0.77	0.43*	1.6	33.5*	5.26*	0.84
CO_2		0.004	<0.001	<0.001	0.013	0.002	0.013	<0.001	0.036	0.357
Soil		<0.001	<0.001	<0.001	0.266	<0.001	<0.001	<0.001	<0.001	0.004
CO_2 × Soil		<0.001	<0.001	<0.001	0.170	0.09	0.208	<0.001	0.017	0.223
Significance at 5-10 cm										
LSD ($P = 0.05$)		1.9	0.05	11.0	0.18	0.49*	1.0	38.9	6.7*	3.5
CO_2		0.729	0.325	0.409	0.116	0.035	<0.001	0.101	0.019	0.06
Soil		<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	0.145
CO_2 × Soil		0.863	0.530	0.399	0.567	0.030	0.500	0.163	0.044	0.813

SOC, soil organic C; DOC, dissolved organic C.

Table S2. The statistical analysis of ANOSIM and ADONIS for CO_2 effect on the bacterial community composition in the 0–5 cm and 5–10 cm layers of the Chromosol, Vertosol and Calcarosol, on which crops had been exposed to either aCO_2 (390 ppm) or eCO_2 (550 ppm) from 2009 to 2015. The test statistic *R* is constrained between the values −1 to 1, and values close to zero represent no difference between CO_2 treatments.

		Chromosol		Vertosol		Calcarosol	
		0–5 cm	5–10 cm	0–5 cm	5–10 cm	0–5 cm	5–10 cm
ANOSIM	Statistic *R*	0.271	0.145	0.031	0.188	0.021	0.063
	p	0.075	0.119	0.462	0.090	0.474	0.698
ADONIS	Statistic *R*	0.230	0.140	0.200	0.240	0.140	0.130
	p	0.092	0.501	0.389	0.205	0.533	0.596