



## Microbial functional genes commonly respond to elevated carbon dioxide

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### ABSTRACT

Atmospheric CO<sub>2</sub> concentration is increasing, largely due to anthropogenic activities. Previous studies of individual free-air CO<sub>2</sub> enrichment (FACE) experimental sites have shown significant impacts of elevated CO<sub>2</sub> (eCO<sub>2</sub>) on soil microbial communities; however, no common microbial response patterns have yet emerged, challenging our ability to predict ecosystem functioning and sustainability in the future eCO<sub>2</sub> environment. Here we analyzed 66 soil microbial communities from five FACE sites, and showed common microbial response patterns to eCO<sub>2</sub>, especially for key functional genes involved in carbon and nitrogen fixation (e.g., *pcc/acc* for carbon fixation, *nifH* for nitrogen fixation), carbon decomposition (e.g., *amyA* and *pulA* for labile carbon decomposition, *mnp* and *lcc* for recalcitrant carbon decomposition), and greenhouse gas emissions (e.g., *mcrA* for methane production, *norB* for nitrous oxide production) across five FACE sites. Also, the relative abundance of those key genes was generally increased and directionally associated with increased biomass, soil carbon decomposition, and soil moisture. In addition, a further literature survey of more disparate FACE experimental sites indicated increased biomass, soil carbon decay, nitrogen fixation, methane and nitrous oxide emissions, plant and soil carbon and nitrogen under eCO<sub>2</sub>. A conceptual framework was developed to link commonly responsive functional genes with ecosystem processes, such as *pcc/acc* vs. soil carbon storage, *amyA/pulA/mnp/lcc* vs. soil carbon decomposition, and *nifH* vs. nitrogen availability, suggesting that such common responses of microbial functional genes may have the potential to predict ecosystem functioning and sustainability in the future eCO<sub>2</sub> environment.

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## 1. Introduction

The concentration of CO<sub>2</sub> in the Earth's atmosphere has increased from 270 ppm in the mid-1800s to > 410 ppm (<https://www.esrl.noaa.gov/gmd/ccgg/trends/>) in 2019 and is predicted to reach 700 ppm by the end of this century (IPCC, 2013), which will accelerate global warming in the future. Previous studies have shown that elevated CO<sub>2</sub> (eCO<sub>2</sub>) generally stimulates plant growth, above- and below-ground biomass, root exudates and fine root turnover, resulting in increased carbon (C) inputs (Ainsworth and Long, 2005; Morgan et al., 2011; Phillips et al., 2011; Reich et al., 2001) and nitrogen (N) inputs (Liang et al., 2016) into soil. Especially, recent studies showed eCO<sub>2</sub> or its induced global warming reduced gut microbiota diversity in a vertebrate ectotherm (Bestion et al., 2017), threatening global human health (Smith and Myers, 2018). However, little is known if such stimulations of plant growth and soil fertility are sustainable in the future eCO<sub>2</sub> environment. As soil microorganisms play critical roles in biogeochemical cycling in terrestrial ecosystems, these ecosystem responses, in turn, can alter the composition and functional traits of soil microbial communities (Carney et al., 2007; Deng et al., 2012; Hayden et al., 2012; He et al., 2012a, 2014, 2010b; Kelley et al., 2011; Lee and Kang, 2016; Tu et al., 2017; Tu et al., 2016; Xiong et al., 2015) as well as their biogeographic distribution (Deng et al., 2016). Therefore, it is important to understand how eCO<sub>2</sub> affects terrestrial ecosystems so that we may accurately predict their impacts on ecosystem functioning in the future CO<sub>2</sub> environment.

The impact of CO<sub>2</sub> on soil microbial communities is considered largely due to indirect effects, such as increased substrate availability and micro-environmental changes under eCO<sub>2</sub> (Adair et al., 2009; Drigo et al., 2013; Feng et al., 2010; He et al., 2014), which have shown highly variable patterns across different terrestrial ecosystems. However, no common patterns have emerged (Carney et al., 2007; Dunbar et al., 2012; Heimann and Reichstein, 2008; Hungate et al., 2009; Jastrow et al., 2005; Kelley et al., 2011), challenging our ability to predict ecosystem functioning under eCO<sub>2</sub>. Such variability may have several possible causes: (i) different ecosystem types and associated characteristics (e.g., C<sub>3</sub> and C<sub>4</sub> plants) differentially respond to eCO<sub>2</sub> (Reich et al., 2018); (ii) increased soil nutrient inputs may allow stochastic processes to play an important role (Zhou et al., 2014); (iii) eCO<sub>2</sub> may lead to progressive nitrogen (N) limitation (Garten et al., 2011; Norby et al., 2010; Reich and Hobbie, 2013); (iv) other environmental factors (e.g., soil moisture) have a large influence on microbial community structure and functions (He et al., 2010b), and an analysis of soil bacterial communities at the Giessen free-air CO<sub>2</sub> enrichment (FACE) experimental site showed that the soil microbiome responded to a soil moisture gradient but not to CO<sub>2</sub> enrichment (Brenzinger et al., 2017; de Menezes et al., 2016); and (v) spatial factors (e.g., soil heterogeneity, geographic distance, plant species and diversity, and ecosystem management) may further drive the divergence of soil microbial communities among disparate ecosystems/sites (Blagodatskaya et al., 2010; Green et al., 2008; Morgan et al., 2011; Weber et al., 2011). Furthermore, some reports indicated that eCO<sub>2</sub> had minimal direct metabolic impacts (Drigo et al., 2008), or that soil microbial community responses were resistant to eCO<sub>2</sub> in a short term (Simonin et al., 2017). Those divergent responses present great challenges for us to predict ecosystem functioning in the future eCO<sub>2</sub> environment. Therefore, it is essential to identify common response patterns of soil microbial communities on a regional or global scale.

Some common changes of ecosystem functioning have been identified in response to eCO<sub>2</sub>. For example, eCO<sub>2</sub> generally increases plant growth, plant productivity and root exudation, leading to increased soil nutrient inputs (He et al., 2014, 2010b; Reich et al., 2001), and alters the soil environment, or microenvironment, such as increased soil moisture (Adair et al., 2009; van Groenigen et al., 2014; Xiong et al., 2015). However, similar common patterns of microbial functions in response to eCO<sub>2</sub> have not been explored yet. Therefore, it is essential for us to identify molecular markers, such as key functional genes

involved in nutrient cycling in response to eCO<sub>2</sub> and use them for potentially predicting ecosystem functions and sustainability.

In this study, we aimed to determine the impact of eCO<sub>2</sub> on soil microbial communities and their common responses across disparate FACE experimental sites for reliably predicting ecosystem functioning and sustainability on a regional, or global scale in the future eCO<sub>2</sub> environment. We hypothesized that there would be common patterns for key soil microbial functional genes (e.g., *nifH* for N<sub>2</sub> fixation, *amyA* and *pulA* for labile carbon degradation, *glx*, *lip mnp* and *lcc* for recalcitrant carbon degradation, *mcrA* for methane production and *norB* for nitrous oxide production) in response to eCO<sub>2</sub> across disparate sites, and those common response patterns would be directionally linked to ecosystem functioning under eCO<sub>2</sub>. To test this core hypothesis, we examined the effects of eCO<sub>2</sub> on the functional potential of 66 soil microbial communities from five FACE experimental sites (BioCON, Duke, SoyFACE, MaizeFACE, and PHACE) in the U.S. (Table S1, Fig. S1) using a comprehensive functional gene array, GeoChip 3.0 (He et al., 2010a). GeoChip targeting key functional processes of biogeochemical cycling of C, N, sulfur (S), phosphorus (P), metals and contaminants in the environment, has been used to analyze the functional diversity, potential and activity of microbial communities from soil, water, extreme and other environments (He et al., 2012b). We also compiled results from meta-analysis of more disparate FACE sites/ecosystems to link observed common microbial responses with ecosystem processes, and developed a conceptual framework to link the common response patterns of microbial functional genes (microbial biomarkers) with ecosystem functioning on a regional or global scale. This study provides new insights into our understanding of microbial responses to eCO<sub>2</sub> and their feedbacks to ecosystem functioning.

## 2. Materials and methods

### 2.1. FACE experimental sites and sampling

This study was conducted at five FACE experimental sites (Fig. S1). The site and sample information included: (i) BioCON (Biodiversity, CO<sub>2</sub> and Nitrogen) at the Cedar Creek Ecosystem Science Reserve, MN (Reich et al., 2001) is a grassland ecosystem and 24 samples/plots were taken from BioCON (12 for aCO<sub>2</sub> and 12 for eCO<sub>2</sub>) of 16 species of native grasses without N addition in July 2007 when this site was exposed to eCO<sub>2</sub> for 10 years; (ii) Duke Forest FACE in Orange County, NC (Lichter et al., 2008) is a forest ecosystem dominated by pine trees and 16 samples/plots (8 each for aCO<sub>2</sub> and eCO<sub>2</sub>) were taken in July 2008 when this site was exposed to eCO<sub>2</sub> for 15 years; (iii) MaizeFACE (Xiong et al., 2015) in Urbana-Champaign, IL is an agroecosystem with maize plants and 8 samples/plots (4 each from aCO<sub>2</sub> and eCO<sub>2</sub>) were taken in July 2007 when it was exposed to eCO<sub>2</sub> for 7 years; (iv) SoyFACE (He et al., 2014) in Urbana-Champaign, IL is an agroecosystem with soybean plants and 8 samples/plots (4 each from aCO<sub>2</sub> and eCO<sub>2</sub>) were taken in October 2008 when they were exposed to eCO<sub>2</sub> for 8 years; and (v) PHACE (Prairie Heating and CO<sub>2</sub> Enrichment) in Cheyenne, WY (Dijkstra et al., 2010) is a mixed-grass prairie semiarid ecosystem dominated by C<sub>4</sub> grasses, C<sub>3</sub> grasses, forbs and sub-shrubs, and 10 samples/plots (5 each from aCO<sub>2</sub> and eCO<sub>2</sub>) were taken in July 2008 when this site was exposed to eCO<sub>2</sub> for only 2 years (Table S1). A total of 66 soil microbial communities at a depth of 0–10 cm or 0–15 cm were analyzed, and they were derived from 98 soil samples as we took three sub-samples from each plot in MaizeFACE and SoyFACE. For experiments with multiple treatments, we only sampled two treatments: ambient CO<sub>2</sub> (aCO<sub>2</sub>) and eCO<sub>2</sub> (550–600 ppm); for experiments with multiple treatments, we only sampled ambient samples for other global change drivers such as nitrogen, ozone, or temperature.

### 2.2. Analysis of soil properties

Soil NO<sub>3</sub>-N and NH<sub>4</sub>-N were extracted with 1 M KCl solution and quantified by a Flow Injection Autoanalyzer (LACHAT, 1994). Soil

organic C and total nitrogen (N) were determined using a LECO Truspec dry combustion carbon analyzer (Nelson and Sommers, 1996).

### 2.3. DNA extraction and purification

For each sample, soil microbial community DNA was extracted and purified as described previously (Zhou et al., 1996). The crude DNA was purified by separation with a low melting agarose gel (0.5%) electrophoresis. The DNA band was excised, melted, extracted with saturated phenol (pH 8.0) and concentrated with 2-butyl alcohol. The DNA quality was measured with an ND-1000 spectrophotometer (Nanodrop, Inc. USA), and the final DNA concentration was quantified using a FLUOstar Optima (BMG Labtech, Jena, Germany). If the DNA quality did not meet our criteria ( $260\text{ nm}/280\text{ nm} > 1.70$ , and  $260\text{ nm}/230\text{ nm} > 1.8$ ), more gel purification was performed. The purified DNA was stored at  $-80\text{ }^{\circ}\text{C}$  until its use.

### 2.4. Functional gene array analysis

We used a comprehensive functional gene array, GeoChip 3.0 to analyze all samples, and it contains about 28,000 probes covering approximately 57,000 gene variants from 292 functional gene families involved in C, N, S and P cycling, energy metabolism, antibiotic resistance, metal resistance and organic contaminant degradation (He et al., 2010a). GeoChip-based hybridization detection is considered quantitative (He et al., 2010b), and details for target preparation, labeling, and GeoChip hybridization as well as data analysis are previously described (He et al., 2012b). Briefly, 50 ng of DNA was used as template for the whole community genome amplification (WCGA) (Wu et al., 2006), and 3.0  $\mu\text{g}$  of amplified DNA was labeled and then hybridized with GeoChip 3.0 at  $45\text{ }^{\circ}\text{C}$  with 50% formamide. The image was processed and spots with a signal to noise ratio (SNR)  $> 2.0$  were considered as positive signals (He and Zhou, 2008), and raw data were pre-processed for further statistical analysis. It is noted that GeoChip hybridization was conducted for 24 samples from SoyFACE and MaizeFACE sites, the data from three sub-samples were combined with their mean values as one sample. GeoChip data are publicly accessible via <http://ieg.ou.edu/4download/>, and other datasets that support the findings of this study are available from the corresponding authors upon request.

### 2.5. Ecosystem data collection and analysis

Ecosystem data from the five sites were measured or collected from the literature (He et al., 2014, 2010b; Lichter et al., 2008; Twine et al., 2013; Xiong et al., 2015), and ecosystem data from a broader range of FACE sites were surveyed from synthetic meta-analyses (Liang et al., 2016; Luo et al., 2006; van Groenigen et al., 2011; van Groenigen et al., 2014).

### 2.6. General strategies for data analysis

As various datasets (e.g., soil properties, ecosystem processes, functional gene abundance) were collected and analyzed in this study, some general strategies were set for data analysis. A meta-analysis approach (response ratio) was used (please see details below). First, although three sub-samples were taken from MaizeFACE and SoyFACE and used for GeoChip hybridization and soil property analysis, the mean value of those three sub-samples was taken for one replicate/sample. Thus, field plots or rings were used for biological replicates, and there are 12, 8, 4, 4, and 5 replicates for each  $\text{CO}_2$  treatment in BioCON, Duke, MaizeFACE, SoyFACE and PHACE, respectively, which was used for within-a-site analysis. Second, for among-all-site (or across-all-site) analysis, the replication was 5 sites, and the value of variables at  $\text{eCO}_2$  was first standardized by  $\text{aCO}_2$  values (e.g., 100%) for each site, and then the  $\text{eCO}_2$  effect was tested among the five sites.

Third, to increase the reliability of all datasets, outliers were removed if a sample had a value greater than two times of standard deviation.

### 2.7. Response ratio analysis of soil property, ecosystem process and functional gene data

The effects of  $\text{eCO}_2$  on soil properties, ecosystem processes, and functional genes were analyzed by computing the response ratio (RR) using the formula described previously (Luo et al., 2006). Especially, for the response ratio analysis of functional gene data, if a given functional gene family, or category contains multiple probes, the sum of all probes in this family, or category was taken for further response ratio analysis. In this study, we used response ratio analysis at two levels: within-a-site and across-all-sites (among-all sites). For within-a-site analysis, the original data were used for both  $\text{aCO}_2$  and  $\text{eCO}_2$  with their replicates for each site (e.g.,  $n = 12$  for BioCON, 8 for Duke, 4 for MaizeFACE and SoyFACE, and 5 for PHACE). For across-all-site analysis, we used  $\text{aCO}_2$  data (the mean value) as 100% to standardize its corresponding  $\text{eCO}_2$  data (the mean value) for each site, and then performed response ratio analysis with the number of sites ( $N = 5$  in this case) as replicates. Based on the across-all-site results, a common response was defined as a significant (e.g., 95% confidence interval) change of functional gene abundance across all sites under  $\text{eCO}_2$  although such a significant change might not be seen within each individual site, while a specific response is defined as non-significant change across all sites under  $\text{eCO}_2$ , but such a change might be significant (e.g., 95% confidence interval) within only one or two sites. If either case is not met, the variable is considered non-responsive. Common responses may be used to predict ecosystem functioning and stability at a global/regional scale (Fig. S2).

### 2.8. Statistical analysis

Preprocessed GeoChip data were further analyzed along with environmental variables by various statistical methods with most of them implemented in the Vegan package in R (R Development Core Team, 2012). Permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the contribution of  $\text{CO}_2$  to microbial community variations by the Adonis function with randomization only implemented within each site to control the effects across all sites (Anderson, 2001). Significance tests were done by F-test based on sequential sums of squares from permutations. Detrended correspondence analysis (DCA) determined the overall functional changes in microbial communities, and three different non-parametric methods were used to test the significance of  $\text{CO}_2$  effects: analysis of similarity (ANOSIM), non-parametric multivariate analysis of variance (PERMANOVA), and multi-response permutation procedure (MRPP) with both Jaccard (non-quantitative) and Bray-Curtis (quantitative) distance matrices as previously described (He et al., 2010b). To elucidate the relationship between soil properties and functional traits of microbial communities, the Mantel test was performed. Soil property data were standardized using the formula:  $s_i = \frac{x_i - \text{Mean}(x_i)}{\text{SD}(x_i)}$ , where  $s_i$  is the standardized value,  $x_i$  is the original value, and  $\text{Mean}(x_i)$  and  $\text{SD}(x_i)$  are the mean value and standard deviation of all  $x$ . The Bray-Curtis distance was used to construct the dissimilarity matrices of microbial communities and environmental variables, respectively.

## 3. Results

### 3.1. Effects of $\text{eCO}_2$ and site on soil properties and ecosystem functions

Analysis of soil properties in the five FACE sites by ANOVA showed that soil nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), total nitrogen (TN), total carbon (TC) and C:N ratio differed significantly by site ( $p < 0.001$ ), but not by  $\text{CO}_2$  treatment ( $p > 0.05$ ), and that a site by  $\text{CO}_2$  interaction

was only significant ( $p < 0.001$ ) for soil  $\text{NO}_3^-$  (Fig. S3). Also, we analyzed the effects of  $\text{eCO}_2$  and site on soil properties and ecosystem functions (Fig. 1) using a response ratio analysis approach (Fig. S4). Overall, our meta-analysis of ecosystem functions showed that  $\text{eCO}_2$  significantly (95% CI) increased plant biomass or net primary production (NPP) (Fig. 1A), and soil C decomposition (Fig. 1C) across those five sites. For soil properties, soil moisture increased (Fig. 1B) and soil nitrate ( $\text{NO}_3\text{-N}$ ) significantly (95% CI) decreased (Fig. 1F), while TC (Fig. 1D), TN (Fig. 1E),  $\text{NH}_4\text{-N}$  (Fig. 1G) or C:N ratio (Fig. 1H) did not significantly change under  $\text{eCO}_2$  across the five sites even though increased trends were observed for TC and C:N ratio. However, for individual sites, a large variation remained. For example, despite a common response pattern in soil C decomposition, this trend was not significant in SoyFACE and MaizeFACE (Fig. 1C). Such among-site differences in soil properties, ecosystem functions and other edaphic factors as well as  $\text{eCO}_2$ -induced changes are expected to influence soil microbial communities and their functional potential.

### 3.2. Overview of soil microbial community responses to $\text{eCO}_2$

Based on all genes ( $N = 10,259$ ) detected by GeoChip 3.0,  $\text{eCO}_2$  significantly ( $p < 0.05$ ) affected soil microbial communities at the whole community composition, functional category, and functional gene family levels as revealed by permutational multivariate analysis of variance (PERMANOVA), especially with the Jaccard method (Tables S2 and S3) although detrended correspondence analysis (DCA) showed that soil microbial communities formed two distinct clusters generally grouped by site or ecosystem rather than by  $\text{CO}_2$  treatment (Fig. 2A). Also, within each site, dissimilarity tests (PERMANOVA, MRPP and ANOSIM) indicated a significant ( $p < 0.05$ )  $\text{CO}_2$  effect in three of the five sites (BioCON, MaizeFACE and SoyFACE but not Duke or PHACE) (Table S2), and this was also seen in the DCA plot (Fig. 2B). In addition, such shifts in soil microbial community structure were significantly correlated (Mantel test,  $p < 0.05$ ) with soil properties, especially total soil C,  $\text{NH}_4^+$  and C:N ratio, and similar results were also observed at the gene category level, and at the gene family level with 18 gene families, such as *amyA*, *ara*, *lip*, *nifH*, *nasA*, *nirS/K*, *ppk*, *ppx* and *dsrA* (Table S4). The results suggested that alterations of nutrients and micro-environment conditions under  $\text{eCO}_2$  had significant effects on the soil microbial community in these ecosystems.

### 3.3. Identification of common and specific patterns of key functional genes in response to $\text{eCO}_2$

We further used response ratio analysis (Fig. S2) to quantify common responses (across all sites) and site-specific responses (within each individual site) of microbial functional genes, soil properties and ecosystem functions under  $\text{eCO}_2$ . Specifically, among 42 functional gene families involved in C, N, S and P cycling examined, common responses to  $\text{eCO}_2$  were observed for 11 functional gene families (e.g., *pcc/acc*, *rbcL*, *amyA*, *pulA*, *vdh*, *lcc*, *mnp*, *mcrA*, *nifH*, *nasA*, *narG*) at a 95% confidence interval (CI), site-specific responses for 14 gene families (e.g., *fhs*, *xylA*, *glx*, *lip*, *gdh*, *nrfA*, *nirS*, *ppk*, *dsrB*, glucoamylase, endoglucanase, exoglucanase, exochitinase and NAG genes), and no significant changes for 17 gene families (e.g., CODH and cellobiase genes, *vanA*, *limEH*, *amoA*, *nirK*, *nosZ*, *ppx*, *dsrA*, and *sox*) (Table 1).

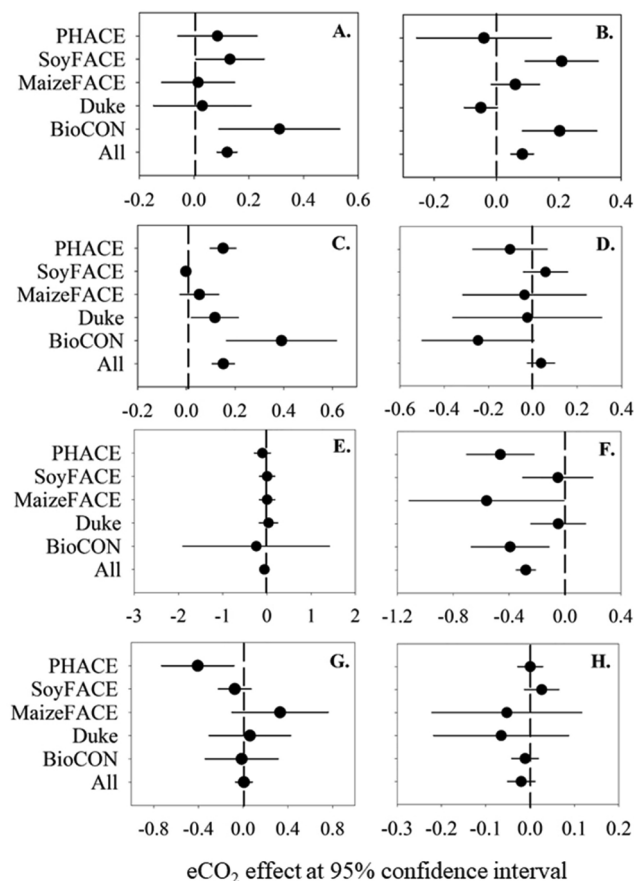
We further linked those commonly responsive functional genes with common directional changes in ecosystem functioning under  $\text{eCO}_2$ , and such ecosystem functional processes, including

$\text{N}_2$  fixation, C decomposition, greenhouse gas emission and others. For example, four key genes involved in C fixation (*pcc/acc*),  $\text{N}_2$  fixation (*nifH*), and labile C degradation (*amyA* and *pulA*) were significantly (95% CI) stimulated by  $\text{eCO}_2$ , and especially, the increase of *nifH* abundance at  $\text{eCO}_2$  was consistently observed in all five sites (Fig. 3A). As recalcitrant C degradation may play more important roles in soil C dynamics (e.g., C input and loss for different C compounds), we also

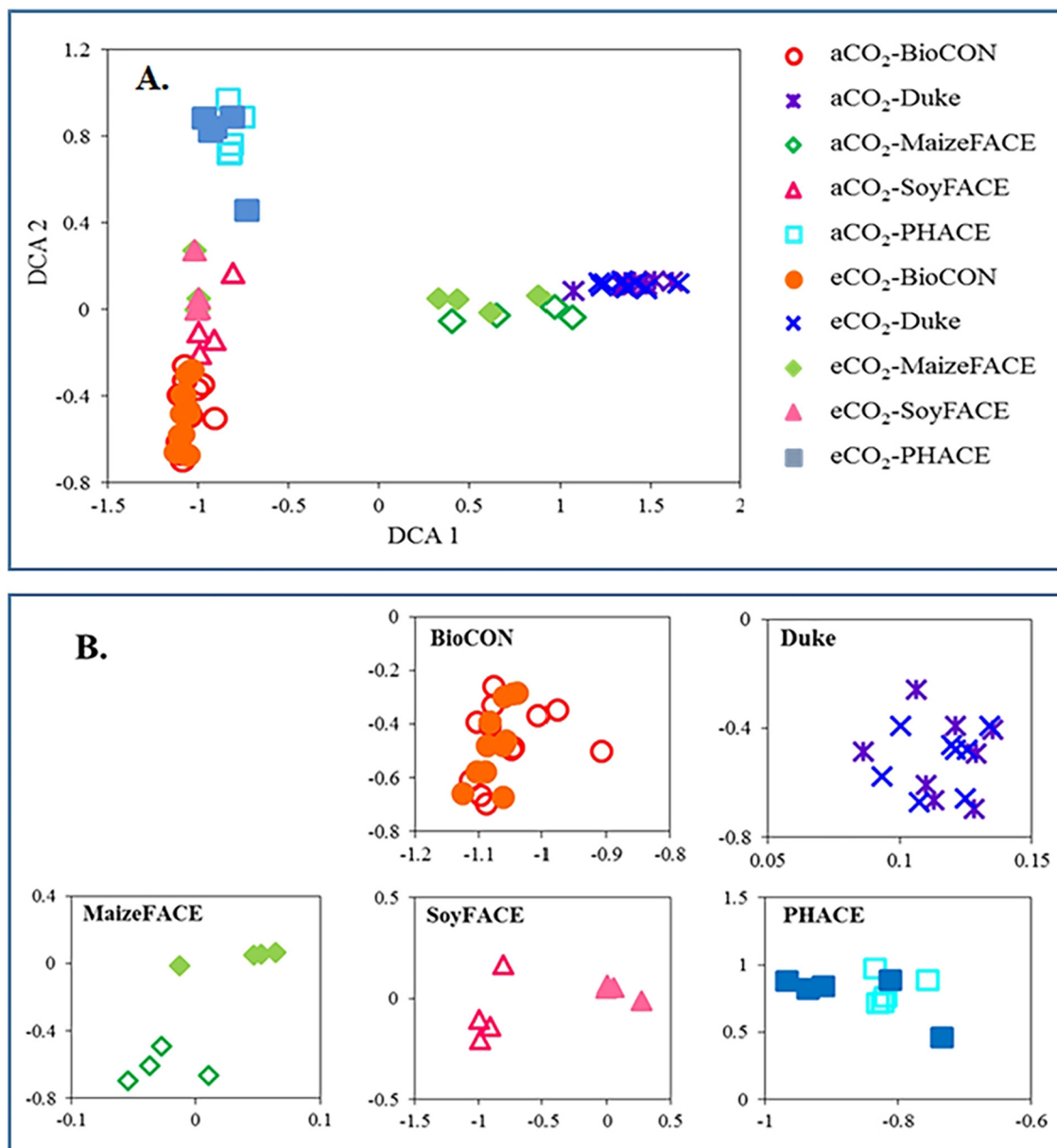
analyzed each of four functional genes (*glx*, *lip*, *mnp* and *lcc*) for lignin degradation, showing that an increase of laccase and manganese peroxidase gene (*lcc* and *mnp*) abundances was common, while glyoxal oxidase and lignin peroxidase genes (*glx* and *lip*) only responded in MaizeFACE, suggesting that  $\text{eCO}_2$  might also increase recalcitrant C degradation potential, especially in some ecosystems like  $\text{C}_4$  plantation ecosystems (Fig. 3B). Additionally, to obtain mechanistic insights into greenhouse gas emissions under  $\text{eCO}_2$ , we analyzed the functional gene markers *mcrA* for  $\text{CH}_4$  generation, *pmoA* for  $\text{CH}_4$  oxidation, *norB* for  $\text{N}_2\text{O}$  generation, and *nosZ* for  $\text{N}_2\text{O}$  reduction, and only found that *mcrA* increased significantly (at 95% CI) as a common response to  $\text{eCO}_2$ , indicating that  $\text{eCO}_2$  might potentially increase  $\text{CH}_4$  emissions, but might not significantly affect  $\text{N}_2\text{O}$  emissions (Fig. 3C). Therefore, our analysis of common responsive functional genes indicated that microbially driven C and N fixation, labile and recalcitrant C decomposition, and  $\text{CH}_4$  emissions could generally increase under  $\text{eCO}_2$ .

### 3.4. Directional linkages between common functional gene abundances and ecosystem functions

To determine whether changes in the abundance of common functional genes are directionally consistent with the changes in ecosystem functions in response to  $\text{eCO}_2$ , we measured ecosystem processes and



**Fig. 1.** Response ratio analysis of the effect of  $\text{eCO}_2$  on soil properties and ecosystem functions at the confidence interval (CI) of 95%. (A) Biomass or net primary production (NPP); (B) Soil moisture; (C) Soil C decomposition; (D) Total soil C; (E) Total soil N; (F) Soil nitrate; (G) Soil ammonium; and H. C:N ratio. If a 95% CI did not overlap with the zero-line, the response was considered significant with a positive  $\text{eCO}_2$  effect on the right or a negative  $\text{eCO}_2$  effect on the left. Details for defining common and specific responses to  $\text{eCO}_2$  and response ratio analysis of within-site and across-all-site samples are described in Fig. S2.



**Fig. 2.** Detrended correspondence analysis (DCA) of all genes ( $N = 10,259$ ) detected by GeoChip 3.0 across all five disparate sites (A) and within each individual site (B), including BioCON, Duke, MaizeFACE, SoyFACE, and PHACE.

compiled historical data from the five FACE sites (He et al., 2014, 2010b; Lichter et al., 2008; Twine et al., 2013; Xiong et al., 2015), and our meta-analysis of soil properties and ecosystem functions revealed significant (95% CI) increases in NPP/biomass, soil C decomposition and soil moisture, a significant decrease in soil  $\text{NO}_3\text{-N}$ , but there was no significant changes in total soil C, N,  $\text{NH}_4\text{-N}$  or C:N ratio under  $\text{eCO}_2$  (Fig. 1).

To obtain a more comprehensive view of linkages between common response functional genes and ecosystem functions, we further synthesized ecosystem data from a broader range of FACE sites/ecosystems (Liang et al., 2016; Luo et al., 2006; van Groenigen et al., 2011, 2014), including a broader range of FACE sites with 21–189 samples for each process, and showed that  $\text{eCO}_2$  increased plant C (~23%) and N (~10%), soil C (~5.6%) and N (~11%), NPP (~20%) and soil C decay (~16%), N fixation (~41%),  $\text{CH}_4$  (~43% in rice fields) and  $\text{N}_2\text{O}$  (~19%) emissions significantly at a 95% CI (Fig. 4). Specifically, when we mapped those commonly responsive functional genes to their responsible ecosystem functions under  $\text{eCO}_2$ , we found that (i) an increase of *nifH* abundance

for N fixation was consistent with increased N fixation and soil N; (ii) an increase of *amyA*, *pulA*, *vdh*, *lcc* and *mnp* abundances for C degradation was consistent with increased soil C decomposition; and (iii) an increase of *mcrA* abundance for methanogenesis was consistent with increased  $\text{CH}_4$  emissions in rice fields (Fig. 4). Also, an increase of *pcc/acc* and *rbcl* abundances for C fixation might be related to increased soil C although it remains unclear about the contribution of microbial C fixation to soil C dynamics. In addition, an increase of *nasA* and *narG* abundances could suggest an enhanced assimilatory nitrate reduction to ammonium (ANRA) under  $\text{eCO}_2$ . Therefore, the abundance of commonly responsive functional genes appeared to be directionally related with ecosystem functions under  $\text{eCO}_2$ .

#### 4. Discussion

Reliable prediction of  $\text{eCO}_2$  impacts on soil microbial communities and ecosystem functioning and sustainability requires knowledge of commonly responsive patterns and variability across disparate

**Table 1**

Common and specific responses of key functional genes across all five sites and within each individual site. Data are presented as eCO<sub>2</sub> effect (%) followed by significance test using response ratio (Luo et al., 2006), and the common and specific responses of genes/enzymes are **bold** in the “All” column, or specific individual site columns, respectively. Details about those functional genes are described previously (He et al., 2010a).

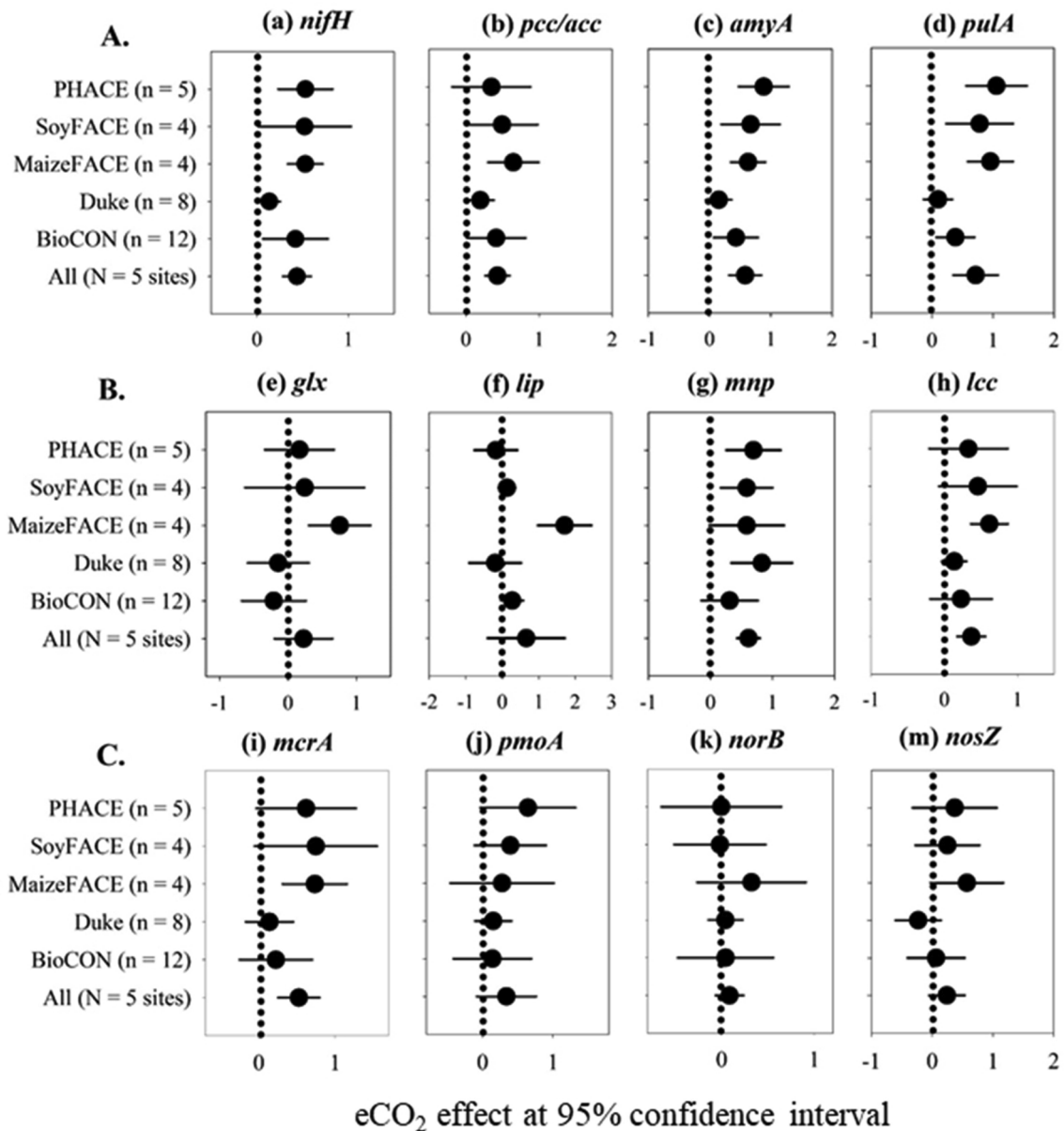
Functional process	Gene/enzyme	CO <sub>2</sub> effect (% change) <sup>a</sup> with response ratio testing of significance					
		All	BioCON	Duke	MaizeFACE	SoyFACE	PHACE
Acetogenesis or C fixation	<i>fhs</i>	46.4*	25.7	-11.5	<b>85.2**</b>	96.3	26.6
	CODH	30.6	41.7	0.7	62.1	33.7	12.9
	<i>pcc/acc</i>	<b>54.0**</b>	51.3**	21.8**	91.4**	64.1**	29.4
	<i>rbcL</i>	<b>50.9**</b>	61.7**	13.5	111.3**	4.9	38.7*
Labile C degradation	Cellobiase	21.1	31.1	6.5	25.3	22.9	16.5
	<i>amyA</i>	<b>79.2**</b>	53.9**	16.4	87.8**	95.5**	58.7**
	Glucosylase	40.7*	17.7	7.9	<b>96.44**</b>	67.9	12.0
	Endoglucanase	43.9	<b>106.6**</b>	3.6	23.9	-26.4	52.7
	Exoglucanase	13.3	-35.0	<b>36.8**</b>	62.8*	12.3	-11.8
	<i>ara</i>	27.5*	<b>64.3**</b>	-5.1	17.4	9.4	33.9
	<i>ara-fungi</i>	15.1	4.5	-13.3	39.0	33.7	10.6
	Endochitinase	12.7	-1.7	-7.5	42.0	33.9	-3.0
	Exochitinase	64.6	-10.7	12.8	58.8*	47.4*	<b>68.2**</b>
	NAG <sup>b</sup>	29.8*	21.8	-11.5	58.6*	48.7	23.8
	<i>xylA</i>	37.3	-3.9	-8.1	<b>99.2**</b>	-5.9	<b>51.3**</b>
	Xylanase	9.9	-11.0	2.0	38.7	23.8	-4.4
	<i>limEH</i>	11.7	-0.1	-19.8	-4.0	17.9	39.1
	<i>pulA</i>	<b>104.7**</b>	46.7**	10.4	160.4**	118.1**	65.3**
	<i>vanA</i>	18.8	3.6	8.5	46.1	-3.9	28.4
<i>vdh</i>	<b>78.9**</b>	33.1	23.0	191.6**	15.6	56.8**	
Recalcitrant C degradation	<i>lcc</i>	<b>44.6**</b>	25.9	14.5	85.4**	58.2*	28.2
	<i>glx</i>	25.3	-18.9	-13.4	<b>112.9**</b>	27.5	15.4
	<i>lip</i>	92.6	30.9	-17.9	<b>452.9**</b>	13.5	-19.4
	<i>mnp</i>	<b>84.7**</b>	36.2	128.6**	79.6	79.4**	49.9**
Methane metabolism	<i>mcrA</i>	<b>69.1**</b>	25.0	15.1	108.4**	111.0	46.3*
	<i>pmoA</i>	40.4*	15.0	16.3	31.8	48.3	47.6*
N cycling	<i>nifH</i>	<b>54.9**</b>	52.3**	14.4*	69.5**	68.4**	41.1**
	<i>amoA</i>	10.3	7.5	10.0	31.5	-18.2	17.3
	<i>nasA</i>	<b>121.7**</b>	71.5**	21.0	165.3**	66.3**	73.9**
	<i>nrjA</i>	57.0*	<b>48.0**</b>	9.4	<b>135.3**</b>	41.3	33.7*
	<i>gdh</i>	41.4	-6.3	0.8	-41.9	<b>134.0**</b>	<b>54.7**</b>
	<i>ureC</i>	26.6	17.3	-4.2	72.2	4.8	30.0
	<i>narG</i>	<b>68.3**</b>	32.4	6.8	128.7**	30.4	58.8**
	<i>nirK</i>	45.9*	45.8	5.1	68.8*	35.7	42.5
	<i>nirS</i>	39.9	19.3	11.4	<b>134.9**</b>	13.4	17.0
	<i>norB</i>	8.9	4.4	4.2	38.0	-1.8	-0.3
P cycling	<i>nosZ</i>	27.5	6.8	-20.6	77.4	28.8	31.1
	<i>ppk</i>	23.2	3.0	8.7	<b>87.6**</b>	-4.5	17.6
	<i>ppx</i>	17.6	7.2	-2.8	68.9*	16.8	-2.2
S cycling	<i>dsrA</i>	20.2	5.8	5.8	47.8	22.1	16.4
	<i>dsrB</i>	34.8*	8.9	<b>44.3**</b>	49.0	11.6	37.7
	<i>sox</i>	15.5	4.2	-6.7	48.6	11.9	16.4

a. CO<sub>2</sub> effect (% change)<sup>a</sup> = (eCO<sub>2</sub> - aCO<sub>2</sub>) \* 100/aCO<sub>2</sub>, where aCO<sub>2</sub> and eCO<sub>2</sub> were the average signal intensities of genes detected at aCO<sub>2</sub> or eCO<sub>2</sub>, respectively; b. NAG: acetylglucosaminidase. Response ratio: \*\*: 95% confidence interval (CI); \*: 90% CI. Details for defining common and specific responses to eCO<sub>2</sub> and response ratio analysis of within-a-site and across-all-site samples are described in Fig. S2.

terrestrial ecosystems. In this study, we identified common responses of microbial functional genes to eCO<sub>2</sub>, and directionally linked the abundance of those functional genes with ecosystem functions or soil properties. The results greatly advance our understanding of microbial responses to eCO<sub>2</sub>, and provide the potential for predicting ecosystem functioning and sustainability by microbial biomarkers, which generally support our core hypothesis.

Our core hypothesis is that there would be common patterns for soil microbial community responses to eCO<sub>2</sub> across disparate FACE sites, and that such common responses would be directionally linked to ecosystem functioning in the future eCO<sub>2</sub> environment. Several possible mechanisms may explain why there are such common patterns for soil microbial communities in response to eCO<sub>2</sub> across disparate sites or ecosystems. First, as a general pattern, eCO<sub>2</sub> stimulates plant growth and plant productivity (Ainsworth and Long, 2005; He et al., 2014, 2010b; Luo et al., 2006; Reich et al., 2001). On one hand, those increases may enhance microbial decomposition and transformation of plant biomass, thus increasing soil nutrients (e.g., soil C, N), which further enhances plant and microbial growth and activity

(Blagodatskaya et al., 2010; Liang et al., 2016; van Groenigen et al., 2014). On the other hand, more soil C and N are taken by plants, especially in agroecosystems where some biomass is moved out of the ecosystems, leading to a decline of nutrients, such as progressive N limitation in soil (Finzi et al., 2006; Johnson, 2006; Norby et al., 2010; Reich and Hobbie, 2013; Reich et al., 2006). To regulate such a decline in soil nutrients, soil microbes may enhance their ability for C and N fixation. For example, many previous studies showed that microbial N fixation or the abundance of N fixation genes increased under eCO<sub>2</sub> (Drake et al., 2011; He et al., 2010b; Li et al., 2017; Luo et al., 2006). If such increased gene abundances are translated to increased N fixation, this may relieve progressive N limitation under eCO<sub>2</sub>. Indeed in this study, we found eCO<sub>2</sub> generally increased the abundance of microbial functional genes, and identified common responses of key genes involved in N fixation (*nifH*), C fixation (*pcc/acc* and *rbcL*), C decomposition (*amyA*, *pulA*, *vdh*, *lcc*, *mnp*), denitrification (*narG*), and ANRA (*nasA*), indicating a general increase in microbial functional potential or activity. Especially, if both N fixation and ANRA were enhanced under eCO<sub>2</sub>, more available N would be provided to plants to relieve

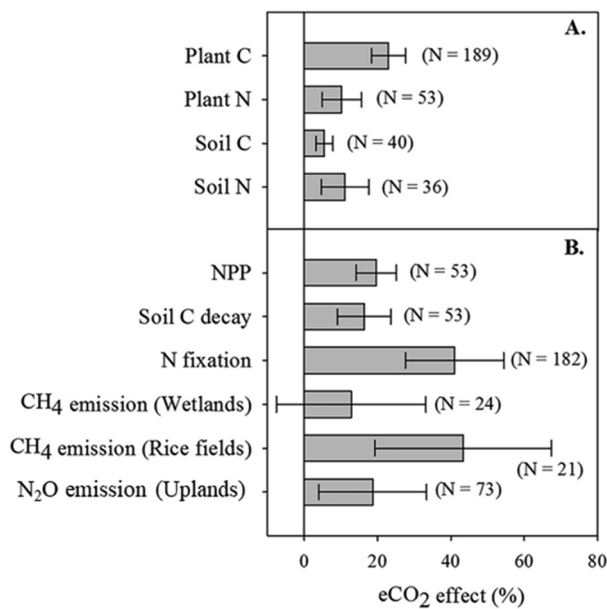


**Fig. 3.** Response ratio analysis of representative key functional genes in response to  $e\text{CO}_2$  across five sites (All) and within each individual site at a 95% confidence interval (CI). (a) *pcc/acc* encoding propionyl-CoA/acetyl-CoA carboxylase for the 3-hydroxypropionate/malyl-CoA cycle (carbon fixation); (b) *nifH* encoding dinitrogenase reductase for microbial  $\text{N}_2$  fixation; (c) *amyA* encoding  $\alpha$ -amylase; (d) *pulA* encoding pullulanase; (e) *glx* encoding glyoxal oxidase; (f) *lip* encoding lignin peroxidase; (g) *mnp* encoding manganese peroxidase, (h) *lcc* encoding laccase/phenol oxidase; (i) *mcrA* encoding the large subunit of methyl coenzyme-M reductase for methanogenesis; (j) *pmoA* encoding particulate methane monooxygenase for methane oxidation; (k) *norB* encoding nitric oxide reductase; and (m) *nosZ* encoding nitrous oxide reductase. If a 95% CI did not overlap with the zero-line, the response was considered significant with a positive  $e\text{CO}_2$  effect on the right or a negative  $e\text{CO}_2$  effect on the left. Details for defining common and specific responses to  $e\text{CO}_2$  and response ratio analysis of within-a-site and across-all-site samples are described in Fig. S2.

progressive N limitation. Furthermore, we found directional linkages between the abundance of common response functional genes and ecosystem functions, which may lead to an increase of NPP, soil C and N across a broad range of FACE experimental sites under  $e\text{CO}_2$ .

Second,  $e\text{CO}_2$  generally stimulates root exudation, a group of small molecules, such as sugars, organics acids and amino acids, and they may stimulate microbial growth and activity, and shape the microbial community diversity, composition and structure (Haichar et al., 2008; Lagomarsino et al., 2007; Phillips et al., 2009, 2011; Wang et al., 2017). For example, a previous study showed that soil microorganisms could rapidly utilize root exudates of rice by a stable isotope probing approach (Yuan et al., 2016), and both field experimental and theoretical modeling analyses showed that root exudates (i.e. C- and N-containing

compounds) affected soil microbial processes in a temperate forest ecosystem by providing more substrates for bio-synthesis of N-rich microbial biomass and exoenzymes (Drake et al., 2013). Also, the significance of root exudates as belowground defense substances has long been recognized, and novel constitutively secreted and inducible phytochemicals may directly repel, inhibit, or kill pathogenic microorganisms in soil (Baetz and Martinoia, 2014). Therefore, although such an array of root exudates are expected to be highly diverse, even differ among different plant species (Bowsher et al., 2016), a trend of  $e\text{CO}_2$ -stimulated root exudation is expected to regulate the composition, structure, function and interaction of soil microbial communities through some unknown mechanisms, which should be further investigated in the future.



**Fig. 4.** Responses of terrestrial ecosystem processes to eCO<sub>2</sub> by response ratio analysis of published datasets across a broader range of FACE sites. The x-axis presents the eCO<sub>2</sub> effect (%) calculated by  $\frac{(eCO_2 - aCO_2) * 100}{aCO_2}$ , where aCO<sub>2</sub> and eCO<sub>2</sub> were the average mean values at aCO<sub>2</sub> or eCO<sub>2</sub>, respectively. N is the total number of replication samples from different FACE sites. The error bars represent 95% confidence intervals (CIs), and a response was considered significant if the 95% CI did not overlap with zero. NPP and soil C decay are from van Groenigen et al. (2014); plant C, soil C, plant N, and soil N are from Luo et al. (2006); CH<sub>4</sub> and N<sub>2</sub>O emissions are from van Groenigen et al. (2011); N fixation is from Liang et al. (2016).

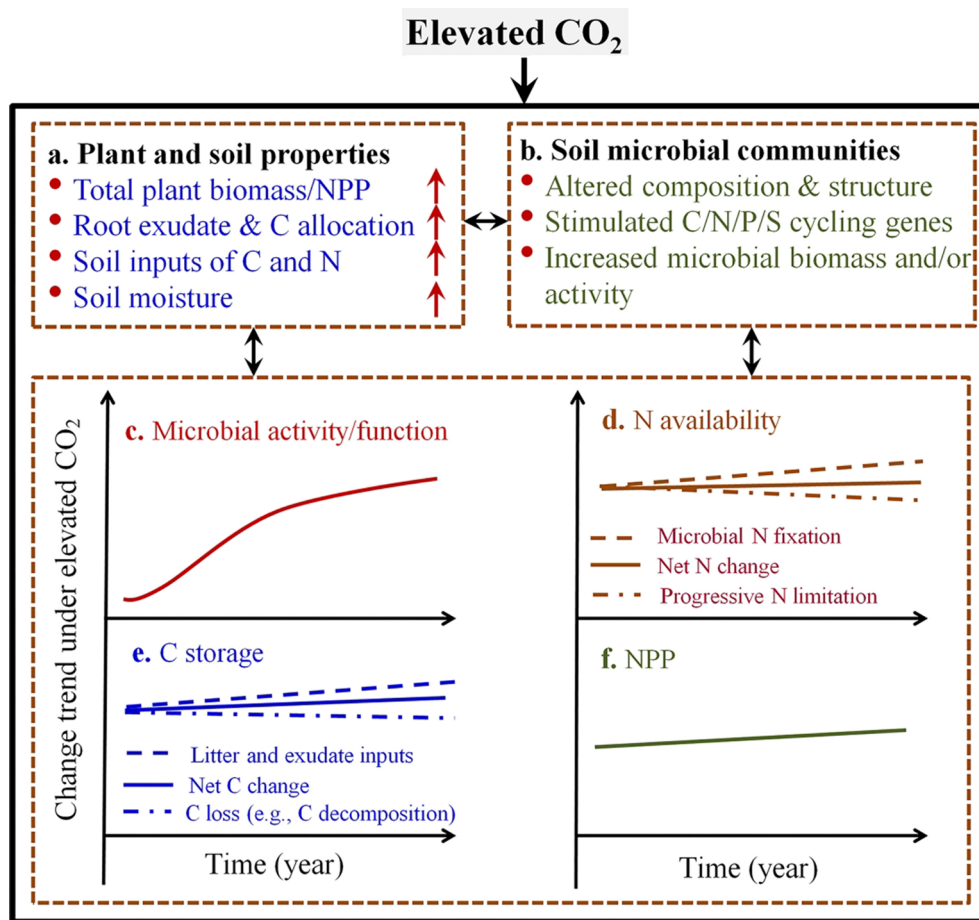
Third, general changes (e.g., soil moisture, soil C, soil N) in the environment or microenvironment may lead to common microbial responses to eCO<sub>2</sub>. One of most significant changes in the soil environment under eCO<sub>2</sub> was an increase of soil moisture (Adair et al., 2009; van Groenigen et al., 2014; Xiong et al., 2015), largely due to reduced stomatal conductance, stomatal density, leaf transpiration, and canopy/ecosystem evapotranspiration under eCO<sub>2</sub> (Xu et al., 2016). For example, a previous study showed that eCO<sub>2</sub> increased soil moisture along with decreased maize evapotranspiration by 7–11% (Hussain et al., 2013). Increased soil moisture generally stimulates microbial growth and activity, especially soil microbes involved in C decomposition and N cycling (He et al., 2010b; van Groenigen et al., 2014), increases anaerobic functional processes (e.g., methanogenesis, denitrification, N fixation), and enhances microbial accessibility to substrates available in soil (Blagodatskaya et al., 2010; He et al., 2014, 2010b; Kelley et al., 2011; van Groenigen et al., 2014). For example, it has been shown that soil microbial activity was consistently enhanced in tallgrass prairie under eCO<sub>2</sub> due to improved soil water conditions (Rice et al., 1994). Also, soil C generally increases under eCO<sub>2</sub>, and it is expected that microbial degradation of C compounds and associated functional genes may be enhanced, which was observed in this study. Although soil N inputs generally increase under eCO<sub>2</sub>, it is expected more N may be also transferred to plants, requiring that the soil microbial community regulates the N cycle. Indeed, this study showed the abundance of functional genes involved in N fixation and ANRA increased, which is directionally linked with increases in soil N and plant N. Therefore, such common responses of soil moisture, soil C and soil N increase may lead to common microbial responses to eCO<sub>2</sub>. Although such direct effects and/or common patterns were not identified in this study, they necessitate further studies in the future.

Based on the above observed common response patterns in this study and our current knowledge, we developed a conceptual

framework to link microbial functional gene abundances with ecosystem functioning and sustainability in response to eCO<sub>2</sub> (Fig. 5). With increases in nutrient inputs, and changes in soil microenvironments, generally increased functional gene abundances were observed under eCO<sub>2</sub>, indicating a general increase in soil microbial potential/activity (Fig. 5a-c). The consistently increased *nifH* abundance indicates a potential for increased microbial N fixation under eCO<sub>2</sub>. As N limitation appears to constrain plant responses to eCO<sub>2</sub> (Garten et al., 2011; Norby et al., 2010; Reich and Hobbie, 2013; Reich et al., 2018), an increase in N fixation may mitigate N limitation and maintain N sustainability in the eCO<sub>2</sub> environment (Fig. 5d). Indeed, we observed increases in N fixation and total soil N under eCO<sub>2</sub> in a broader range of FACE sites of the literature survey. Although this study was focused on the effect of eCO<sub>2</sub> on free-living N<sub>2</sub> fixers with bulk soil samples, previous studies also showed that eCO<sub>2</sub> could increase symbiotic N<sub>2</sub> fixation and amplify the benefit of N<sub>2</sub> fixation in legumes, resulting in more N inputs into grassland ecosystems (Rogers et al., 2009; Soussana and Hartwig, 1996). Also, increased abundances of key C degradation genes may indicate an increased potential for soil C degradation, providing more substrates and nutrients for soil microbial growth and activity, consequently constraining soil C storage and maintaining its sustainability under eCO<sub>2</sub> (Fig. 5e), which is consistent with a previous meta-analysis (van Groenigen et al., 2014). The abundance of key genes for both labile C (e.g., *amyA*, *pulA*) and recalcitrant C (e.g., *mnp*, *lcc*) degradation consistently increased at eCO<sub>2</sub>, which could increase soil C decomposition due to increased carbon inputs (litter and root exudation), microbial activity for carbon decomposition and microbial accessibility of substrates (Kelley et al., 2011; Phillips et al., 2011; Reich et al., 2001; van Groenigen et al., 2014) as observed in the five sites and a broader range of sites, leading to partial loss of eCO<sub>2</sub>-induced soil C inputs. Soil C storage and sustainability largely depend on the balance between soil C inputs and soil C loss (e.g., decomposition) as well as their accessibility by microorganisms. Soil C did increase under eCO<sub>2</sub> in our literature survey, indicating a possible C sink in the future eCO<sub>2</sub> environment. In addition, an increase of *mcrA* abundance at eCO<sub>2</sub> was observed, which appeared to be consistent with an increased trend of CH<sub>4</sub> emissions in rice fields in our literature survey, and a recent study, showing that eCO<sub>2</sub> promoted methanogenesis and suppressed methane oxidation in rice paddy soils (Okubo et al., 2015). As soil microbial activity and total soil C and N increase, NPP is expected to continuously increase and maintain its sustainability in the future eCO<sub>2</sub> environment (Fig. 5f). As microbial functional genes were found to be able to predict N<sub>2</sub>O concentrations and environmental contamination in groundwater (He et al., 2018) and in soil (Orellana et al., 2014), commonly responsive functional genes (e.g., *nifH*, *amyA*, *pulA*, *mnp*, *lcc*, *mcrA*) identified in this study and new data from their deployment should be valuable for constraining microbial contributions to ecosystem processes, potentially for predicting ecosystem functioning and sustainability. However, we should mention a couple of important points for future studies. First, we did not see an increased abundance of functional genes involved in N<sub>2</sub>O emissions in this study, while an increase of N<sub>2</sub>O emissions under eCO<sub>2</sub> was observed in upland soils (van Groenigen et al., 2011), indicating a possible lack of directional linkage between N<sub>2</sub>O metabolic genes and N<sub>2</sub>O emissions. Second, it is noted that a significant increase in soil C and soil N was not seen in the five FACE sites but it was observed in our broad literature survey, implying that clearer and more reliable patterns may be obtained with more FACE sites and more samples. Third, an explicit incorporation of microbial data into global change models remains challenging.

In summary, this study provides novel insights into our understanding of soil microbial community responses and their feedbacks to eCO<sub>2</sub> from microbial functional ecology perspectives. We identified common response patterns of microbial functional genes under eCO<sub>2</sub>, and directionally linked their abundance changes with the changes in soil properties, ecosystem functioning, which is the first step to use a set of potential molecular biomarkers of global change for predicting





**Fig. 5.** A conceptual framework for linking the soil microbial community and ecosystem functioning in response to eCO<sub>2</sub>. (a) Based on previous studies, eCO<sub>2</sub> generally increases plant biomass/net primary productivity (NPP) and root exudation, and alters the soil microenvironment (e.g., soil moisture), thus modifying the soil microbial community structure and function. (b) In response to the changes in plant and soil properties, soil microbial communities alter their community composition and structure, stimulate C, N, S and P cycling genes, and provide more nutrients for microbial growth and activity, thus maintaining plant growth and regulating ecosystem functional processes. (c) Based on our results from this study and the current knowledge, it is predicted that soil microbial community function and activity increase at eCO<sub>2</sub>. (d) Soil net N may remain increasing as microbial N fixation may positively balance the progressive N limitation and other N transformation processes at eCO<sub>2</sub>. (e) Soil C storage may remain stably increasing by increased C inputs from litter and root exudates despite offset by increased C decomposition at eCO<sub>2</sub>. (f) A stably increased NPP may be sustained in the future eCO<sub>2</sub> environment when soil C and N stably increase in a long-term run. Solid lines indicate predicted microbial activity/function in (c), net N change in (d), net C change in (e), and NPP in (f), while dashed lines show increased microbial N fixation against progressive N limitation (a decrease of N availability at eCO<sub>2</sub>, dash-dot lines) in (d), and increased C inputs (e.g., litter and root exudation) offset by C loss (an increase of C decomposition at eCO<sub>2</sub>, dash-dot lines) in (e).

ecosystem functioning and sustainability on a regional or global scale. Also, we developed a conceptual framework to link those functional genes with ecosystem functioning and sustainability across a broader range of FACE sites, suggesting that soil N and C, consequently NPP may continuously increase and maintain their ecosystem sustainability in the future eCO<sub>2</sub> environment. This study has important implications for future efforts to inform, constrain, validate, and/or develop global change models.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Author contributions

All authors contributed to the data set, discussed the results and commented on the manuscript. J.Z. and Z.H. designed this study; J.X., H.Y., M.X. and J.Li carried out GeoChip and soil property analysis; Y.D., K.X., J.Liang and B.W. did data analysis; Q.Y. and L.W. provided resources for experiments and sampling; Z.H., Y.D. and M.X. wrote this paper with help from S.S., Y.C., J.D.V.N., S.E.H., P.B.R., C.W.S., A.D.K., E.P., M.W., Y.L., Q.Y. and J.Z.

#### Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2020.106068>.

#### References

Adair, E.C., Reich, P.B., Hobbie, S.E., Knops, J.M.H., 2009. Interactive effects of time, CO<sub>2</sub>, N, and diversity on total belowground carbon allocation and ecosystem carbon

- storage in a grassland community. *Ecosystems* 12, 1037–1052.
- Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO<sub>2</sub> enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO<sub>2</sub>. *New Phytol.* 165, 351–372.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46.
- Baetz, U., Martinioia, E., 2014. Root exudates: the hidden part of plant defense. *Trends Plant Sci.* 19, 90–98.
- Bestion, E., Jacob, S., Zinger, L., Di Gesu, L., Richard, M., White, J., Cote, J., 2017. Climate warming reduces gut microbiota diversity in a vertebrate ectotherm. *Nat. Ecol. Evol.* 1, 0161.
- Blagodatskaya, E., Blagodatsky, S., Dorodnikov, M., Kuzyakov, Y., 2010. Elevated atmospheric CO<sub>2</sub> increases microbial growth rates in soil: results of three CO<sub>2</sub> enrichment experiments. *Glob. Change Biol.* 16, 836–848.
- Bowsher, A.W., Ali, R., Harding, S.A., Tsai, C.-J., Donovan, L.A., 2016. Evolutionary divergences in root exudate composition among ecologically-contrasting *Helianthus* species. *PLoS ONE* 11, e0148280.
- Brenzinger, K., Kujala, K., Horn, M.A., Moser, G., Guillet, C., Kammann, C., Müller, C., Braker, G., 2017. Soil conditions rather than long-term exposure to elevated CO<sub>2</sub> affect soil microbial communities associated with N-cycling. *Front. Microbiol.* 8, art1976.
- Carney, M.C., Hungate, B.A., Drake, B.G., Megonigal, J.P., 2007. Altered soil microbial community at elevated CO<sub>2</sub> leads to loss of soil carbon. *PNAS* 104, 4990–4995.
- de Menezes, A.B., Müller, C., Clipson, N., Doyle, E., 2016. The soil microbiome at the GiFACE experiment responds to a moisture gradient but not to CO<sub>2</sub> enrichment. *Microbiology* 162, 1572–1582.
- Deng, Y., He, Z., Xiong, J., Yu, H., Xu, M., Hobbie, S.E., Reich, P.B., Schadt, C.W., Kent, A., Pendall, E., Wallenstein, M., Zhou, J., 2016. Elevated carbon dioxide accelerates the spatial turnover of soil microbial communities. *Glob. Change Biol.* 22, 957–964.
- Deng, Y., He, Z., Xu, M., Qin, Y., Van Nostrand, J.D., Wu, L., Roe, B.A., Wiley, G., Hobbie, S.E., Reich, P.B., Zhou, J., 2012. Elevated carbon dioxide alters the structure of soil microbial communities. *Appl. Environ. Microbiol.* 78, 2991–2995.
- Dijkstra, F.A., Blumenthal, D., Morgan, J.A., Pendall, E., Carrillo, Y., Follett, R.F., 2010. Contrasting effects of elevated CO<sub>2</sub> and warming on nitrogen cycling in a semiarid grassland. *New Phytol.* 187, 426–437.
- Drake, J.E., Darby, B.A., Giasson, M.A., Kramer, M.A., Phillips, R.P., Finzi, A.C., 2013. Stoichiometry constrains microbial response to root exudation- insights from a model and a field experiment in a temperate forest. *Biogeosciences* 10, 821–838.
- Drake, J.E., Gallet-Budynek, A., Hofmockel, K.S., Bernhardt, E.S., Billings, S.A., Jackson, R.B., Johnsen, K.S., Lichter, J., McCarthy, H.R., McCormack, M.L., 2011. Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO<sub>2</sub>. *Ecol. Lett.* 14, 349–357.
- Drigo, B., Kowalchuk, G., van Veen, J., 2008. Climate change goes underground: effects of elevated atmospheric CO<sub>2</sub> on microbial community structure and activities in the rhizosphere. *Biol. Fertil. Soils* 44, 667–679.
- Drigo, B., Kowalchuk, G.A., Knapp, B.A., Pijl, A.S., Boschker, H.T.S., van Veen, J.A., 2013. Impacts of 3 years of elevated atmospheric CO<sub>2</sub> on rhizosphere carbon flow and microbial community dynamics. *Glob. Change Biol.* 19, 621–636.
- Dunbar, J., Eichorst, S.A., Gallegos-Graves, L.V., Silva, S., Xie, G., Hengartner, N.W., Evans, R.D., Hungate, B.A., Jackson, R.B., Megonigal, J.P., Schadt, C.W., Vilgalys, R., Zak, D.R., Kuske, C.R., 2012. Common bacterial responses in six ecosystems exposed to 10 years of elevated atmospheric carbon dioxide. *Environ. Microbiol.* 14, 1145–1158.
- Feng, X., Simpson, A.J., Schlesinger, W.H., Simpson, M.J., 2010. Altered microbial community structure and organic matter composition under elevated CO<sub>2</sub> and N fertilization in the duke forest. *Glob. Change Biol.* 16, 2104–2116.
- Finzi, A.C., Moore, D.J., DeLucia, E.H., Lichter, J., Hofmockel, K.S., Jackson, R.B., Kim, H.-S., Matamala, R., McCarthy, H.R., Oren, R., 2006. Progressive nitrogen limitation of ecosystem processes under elevated CO<sub>2</sub> in a warm-temperate forest. *Ecology* 87, 15–25.
- Garten, C.T., Iversen, C.M., Norby, R.J., 2011. Litterfall <sup>15</sup>N abundance indicates declining soil nitrogen availability in a free-air CO<sub>2</sub> enrichment experiment. *Ecology* 92, 133–139.
- Green, J.L., Bohannan, B.J.M., Whitaker, R.J., 2008. Microbial Biogeography: From taxonomy to traits. *Science* 320, 1039–1043.
- Haichar, F.Z., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., Heulin, T., Achouak, W., 2008. Plant host habitat and root exudates shape soil bacterial community structure. *ISME J.* 2, 1221.
- Hayden, H.L., Mele, P.M., Bougoure, D.S., Allan, C.Y., Norng, S., Piceno, Y.M., Brodie, E.L., DeSantis, T.Z., Andersen, G.L., Williams, A.L., Hovenden, M.J., 2012. Changes in the microbial community structure of bacteria, archaea and fungi in response to elevated CO<sub>2</sub> and warming in an Australian native grassland soil. *Environ. Microbiol.* 14, 3081–3096.
- He, Z., Deng, Y., Van Nostrand, J.D., Tu, Q., Xu, M., Hemme, C.L., Li, X., Wu, L., Gentry, T.J., Yin, Y., Liebich, J., Hazen, T.C., Zhou, J.Z., 2010a. GeoChip 3.0 as a high-throughput tool for analyzing microbial community composition, structure and functional activity. *ISME J.* 4, 1167–1179.
- He, Z., Piceno, Y., Deng, Y., Xu, M., Lu, Z., DeSantis, T., Andersen, G., Hobbie, S.E., Reich, P.B., Zhou, J.Z., 2012a. The phylogenetic composition and structure of soil microbial communities shifts in response to elevated carbon dioxide. *ISME J.* 6, 259–272.
- He, Z., Van Nostrand, J.D., Zhou, J.Z., 2012b. Applications of functional gene microarrays for profiling microbial communities. *Curr. Opin. Biotechnol.* 23, 460–466.
- He, Z., Xiong, J., Kent, A.D., Deng, Y., Xue, K., Wang, G., Wu, L., Van Nostrand, J.D., Zhou, J., 2014. Distinct responses of soil microbial communities to elevated CO<sub>2</sub> and O<sub>3</sub> in a soybean agro-ecosystem. *ISME J.* 8, 714–726.
- He, Z., Xu, M., Deng, Y., Kang, S., Kellogg, L., Wu, L., Van Nostrand, J.D., Hobbie, S.E., Reich, P.B., Zhou, J., 2010b. Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO<sub>2</sub>. *Ecol. Lett.* 13, 564–575.
- He, Z., Zhang, P., Wu, L., Rocha, A.M., Tu, Q., Shi, Z., Wu, B., Qin, Y., Wang, J., Yan, Q., 2018. Microbial functional gene diversity predicts groundwater contamination and ecosystem functioning. *mBio* 9, e02435–02417.
- He, Z., Zhou, J., 2008. Empirical evaluation of a new method for calculating signal-to-noise ratio for microarray data analysis. *Appl. Environ. Microbiol.* 74, 2957–2966.
- Heimann, M., Reichstein, M., 2008. Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* 451, 289–292.
- Hungate, B.A., Van Groenigen, K.-J., Six, J., Jastrow, J.D., Luo, Y., De Graaff, M.-A., Van Kessel, C., Osenberg, C.W., 2009. Assessing the effect of elevated carbon dioxide on soil carbon: a comparison of four meta-analyses. *Glob. Change Biol.* 15, 2020–2034.
- Hussain, M.Z., VanLooke, A., Siebers, M.H., Ruiz-Vera, U.M., Cody Markelz, R.J., Leakey, A.D.B., Ort, D.R., Bernacchi, C.J., 2013. Future carbon dioxide concentration decreases canopy evapotranspiration and soil water depletion by field-grown maize. *Glob. Change Biol.* 19, 1572–1584.
- IPCC, 2013. Intergovernmental Panel on Climate Change. *Climate Change 2013: The Physical Science Basis: The Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge Univ. Press).
- Jastrow, J.D., Miller, R.M., Matamala, R., Norby, R.J., Boutton, T.W., 2005. Elevated atmospheric carbon dioxide increases soil carbon. *Glob. Change Biol.* 11, 2057–2064.
- Johnson, D.W., 2006. Progressive N limitation in forests: review and implications for long-term responses to elevated CO<sub>2</sub>. *Ecology* 87, 64–75.
- Kelley, A.M., Fay, P.A., Polley, H.W., Gill, R.A., Jackson, R.B., 2011. Atmospheric CO<sub>2</sub> and soil extracellular enzyme activity: a meta-analysis and CO<sub>2</sub> gradient experiment. *Ecosphere* 2, art96.
- LACHAT, 1994. *QuickChem Method 12-107-04-1-B. Milwaukee, WI: LACHAT Instrument.*
- Lagomasino, A., Knapp, B., Moscatelli, M., De Angelis, P., Grego, S., Insam, H., 2007. Structural and functional diversity of soil microbes is affected by elevated [CO<sub>2</sub>] and N addition in a poplar plantation. *J. Soils Sediments* 7, 399–405.
- Lee, S.-H., Kang, H., 2016. Elevated CO<sub>2</sub> causes a change in microbial communities of rhizosphere and bulk soil of salt marsh system. *Appl. Soil Ecol.* 108, 307–314.
- Li, Y., Yu, Z., Liu, X., Mathesius, U., Wang, G., Tang, C., Wu, J., Liu, J., Zhang, S., Jin, J., 2017. Elevated CO<sub>2</sub> increases nitrogen fixation at the reproductive phase contributing to various yield responses of soybean cultivars. *Front. Plant Sci.* 8, 1546.
- Liang, J., Qi, X., Souza, L., Luo, Y., 2016. Processes regulating progressive nitrogen limitation under elevated carbon dioxide: a meta-analysis. *Biogeosciences* 13, 2689–2699.
- Lichter, J., Billings, S.A., Ziegler, S.E., Gaidh, D., Ryals, R., Finzi, A.C., Jackson, R.B., Stemmler, E.A., Schlesinger, W.H., 2008. Soil carbon sequestration in a pine forest after 9 years of atmospheric CO<sub>2</sub> enrichment. *Glob. Change Biol.* 14, 2910–2922.
- Luo, Y., Hui, D., Zhang, D., 2006. Elevated CO<sub>2</sub> stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. *Ecology* 87, 53–63.
- Morgan, J.A., LeCain, D.R., Pendall, E., Blumenthal, D.M., Kimball, B.A., Carrillo, Y., Williams, D.G., Heisler-White, J., Dijkstra, F.A., West, M., 2011. C<sub>4</sub> grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature* 476, 202–205.
- Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (Eds.), *Methods of Soil Analysis. Soil Science Society of America, Madison, WI.*
- Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E., McMurtrie, R.E., 2010. CO<sub>2</sub> enhancement of forest productivity constrained by limited nitrogen availability. *PNAS* 107, 19368–19373.
- Okubo, T., Liu, D., Tsurumaru, H., Ikeda, S., Asakawa, S., Tokida, T., Tago, K., Hayatsu, M., Aoki, N., Ishimaru, K., Ujiie, K., Usui, Y., Nakamura, H., Sakai, H., Hayashi, K., Hasegawa, T., Minamisawa, K., 2015. Elevated atmospheric CO<sub>2</sub> levels affect community structure of rice root-associated bacteria. *Front. Microbiol.* 6, 136.
- Orellana, L.H., Rodriguez-R, L.M., Higgins, S., Chee-Sanford, J.C., Sanford, R.A., Ritalhti, K.M., Löffler, F.E., Konstantinidis, K.T., 2014. Detecting nitrous oxide reductase (nosZ) genes in soil metagenomes: method development and implications for the nitrogen cycle. *mBio* 5, e01193-01114.
- Phillips, R.P., Bernhardt, E.S., Schlesinger, W.H., 2009. Elevated CO<sub>2</sub> increases root exudation from loblolly pine (*Pinus taeda*) seedlings as an N-mediated response. *Tree Physiol.* 29, 1513–1523.
- Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO<sub>2</sub> fumigation. *Ecol. Lett.* 14, 187–194.
- R Development Core Team, 2012. *R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing* (<http://www.R-project.org>). Vienna, Austria.
- Reich, P.B., Hobbie, S.E., 2013. Decade-long soil nitrogen constraint on the CO<sub>2</sub> fertilization of plant biomass. *Nat. Clim. Change* 3, 278–282.
- Reich, P.B., Hobbie, S.E., Lee, T., Ellsworth, D.S., West, J.B., Tilman, D., Knops, J.M., Naem, S., Trost, J., 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO<sub>2</sub>. *Nature* 440, 922–925.
- Reich, P.B., Hobbie, S.E., Lee, T.D., Pastore, M.A., 2018. Unexpected reversal of C<sub>3</sub> versus C<sub>4</sub> grass response to elevated CO<sub>2</sub> during a 20-year field experiment. *Science* 360, 317–320.
- Reich, P.B., Knops, J., Tilman, D., Craine, J., Ellsworth, D., Tjoelker, M., Lee, T., Wedin, D., Naem, S., Bahauddin, D., Hendrey, G., Jose, S., Wrage, K., Goth, J., Bengtson, W., 2001. Plant diversity enhances ecosystem responses to elevated CO<sub>2</sub> and nitrogen deposition. *Nature* 410, 809–812.
- Rice, C.W., Garcia, F.O., Hampton, C.O., Owensby, C.E., 1994. Soil microbial response in tallgrass prairie to elevated CO<sub>2</sub>. *Plant Soil* 165, 67–74.

- Rogers, A., Ainsworth, E.A., Leakey, A.D.B., 2009. Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? *Plant Physiol.* 151, 1009–1016.
- Simonin, M., Nunan, N., Bloor, J.M.G., Pouteau, V., Niboyet, A., 2017. Short-term responses and resistance of soil microbial community structure to elevated CO<sub>2</sub> and N addition in grassland mesocosms. *FEMS Microbiol. Lett.* 364, fnx077.
- Smith, M.R., Myers, S.S., 2018. Impact of anthropogenic CO<sub>2</sub> emissions on global human nutrition. *Nat. Clim. Change* 8, 834–839.
- Soussana, J.A., Hartwig, U.A., 1996. The effects of elevated CO<sub>2</sub> on symbiotic N<sub>2</sub> fixation: a link between the carbon and nitrogen cycles in grassland ecosystems. *Plant Soil* 187, 321–332.
- Tu, Q., He, Z., Wu, L., Xue, K., Xie, G., Chain, P., Reich, P.B., Hobbie, S.E., Zhou, J., 2017. Metagenomic reconstruction of nitrogen cycling pathways in a CO<sub>2</sub>-enriched grassland ecosystem. *Soil Biol. Biochem.* 106, 99–108.
- Tu, Q., Zhou, X., He, Z., Xue, K., Wu, L., Reich, P., Hobbie, S., Zhou, J., 2016. The diversity and co-occurrence patterns of N<sub>2</sub>-fixing communities in a CO<sub>2</sub>-enriched grassland ecosystem. *Microb. Ecol.* 71, 604–615.
- Twine, T.E., Bryant, J.J., Richter, K., Bernacchi, C.J., McConnaughay, K.D., Morris, S.J., Leakey, A.D.B., 2013. Impacts of elevated CO<sub>2</sub> concentration on the productivity and surface energy budget of the soybean and maize agroecosystem in the Midwest U.S. *Glob. Change Biol.* 19, 2838–2852.
- van Groenigen, K.J., Osenberg, C.W., Hungate, B.A., 2011. Increased soil emissions of potent greenhouse gases under increased atmospheric CO<sub>2</sub>. *Nature* 475, 214–216.
- van Groenigen, K.J., Qi, X., Osenberg, C.W., Luo, Y., Hungate, B.A., 2014. Faster decomposition under increased atmospheric CO<sub>2</sub> limits soil carbon storage. *Science* 344, 508–509.
- Wang, P., Marsh, E.L., Ainsworth, E.A., Leakey, A.D.B., Sheflin, A.M., Schachtman, D.P., 2017. Shifts in microbial communities in soil, rhizosphere and roots of two major crop systems under elevated CO<sub>2</sub> and O<sub>3</sub>. *Sci. Rep.* 7, 15019.
- Weber, C.F., Zak, D.R., Hungate, B.A., Jackson, R.B., Vilgalys, R., Evans, R.D., Schadt, C.W., Megonigal, J.P., Kuske, C.R., 2011. Responses of soil cellulolytic fungal communities to elevated atmospheric CO<sub>2</sub> are complex and variable across five ecosystems. *Environ. Microbiol.* 13, 2778–2793.
- Wu, L., Liu, X., Schadt, C.W., Zhou, J., 2006. Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. *Appl. Environ. Microbiol.* 72, 4931–4941.
- Xiong, J., He, Z., Shi, S., Kent, A., Deng, Y., Wu, L., Van Nostrand, J.D., Zhou, J., 2015. Elevated CO<sub>2</sub> shifts the functional structure and metabolic potentials of soil microbial communities in a C4 agroecosystem. *Soil Biol. Biochem.* 88, 931–941.
- Xu, Z., Jiang, Y., Jia, B., Zhou, G., 2016. Elevated-CO<sub>2</sub> response of stomata and its dependence on environmental factors. *Front. Plant Sci.* 7, 657.
- Yuan, H., Zhu, Z., Liu, S., Ge, T., Jing, H., Li, B., Liu, Q., Lynn, T.M., Wu, J., Kuzyakov, Y., 2016. Microbial utilization of rice root exudates: <sup>13</sup>C labeling and PLFA composition. *Biol. Fertil. Soils* 52, 615–627.
- Zhou, J., Bruns, M.A., Tiedje, J.M., 1996. DNA recovery from soils of diverse composition. *Appl. Environ. Microbiol.* 62, 316–322.
- Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J.D., Yang, Y., He, Z., Wu, L., Stahl, D.A., Hazen, T.C., Tiedje, J.M., Arkin, A.P., 2014. Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. *PNAS* 111, E836–E845.