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Microbial functional genes commonly respond to elevated carbon dioxide

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ABSTRACT

Atmospheric CO_2 concentration is increasing, largely due to anthropogenic activities. Previous studies of individual free-air CO_2 enrichment (FACE) experimental sites have shown significant impacts of elevated CO_2 (eCO_2) 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_2 environment. Here we analyzed 66 soil microbial communities from five FACE sites, and showed common microbial response patterns to eCO_2, 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_2. 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_2 environment.

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1. Introduction

The concentration of CO_2 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_2 (eCO_2) 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_2 or its induced global warming reduced gut microbiota diversity in a vertebrate ectotherm (Bestion et al., 2017), threating 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_2 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_2 affects terrestrial ecosystems so that we may accurately predict their impacts on ecosystem functioning in the future CO_2 environment.

The impact of CO_2 on soil microbial communities is considered largely due to indirect effects, such as increased substrate availability and micro-environmental changes under eCO_2 (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_2. Such variability may have several possible causes: (i) different ecosystem types and associated characteristics (e.g., C_3 and C_4 plants) differentially respond to eCO_2 (Reich et al., 2018); (ii) increased soil nutrient inputs may allow stochastic processes to play an important role (Zhou et al., 2014); (iii) eCO_2 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_2 enrichment (FACE) experimental site showed that the soil microbiome responded to a soil moisture gradient but not to CO_2 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_2 had minimal direct metabolic impacts (Drigo et al., 2008), or that soil microbial community responses were resistant to eCO_2 in a short term (Simonin et al., 2017). Those divergent responses present great challenges for us to predict ecosystem functioning in the future eCO_2 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_2. For example, eCO_2 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_2 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_2 and use them for potentially predicting ecosystem functions and sustainability.

In this study, we aimed to determine the impact of eCO_2 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_2 environment. We hypothesized that there would be common patterns for key soil microbial functional genes (e.g., *nifH* for N_2 fixation, *amyA* and *pulA* for liable 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_2 across disparate sites, and those common response patterns would be directionally linked to ecosystem functioning under eCO_2. To test this core hypothesis, we examined the effects of eCO_2 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_2 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_2 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_2 and 12 for eCO_2) of 16 species of native grasses without N addition in July 2007 when this site was exposed to eCO_2 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_2 and eCO_2) were taken in July 2008 when this site was exposed to eCO_2 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_2 and eCO_2) were taken in July 2007 when it was exposed to eCO_2 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_2 and eCO_2) were taken in October 2008 when they were exposed to eCO_2 for 8 years; and (v) PHACE (Prairie Heating and CO_2 Enrichment) in Cheyenne, WY (Dijkstra et al., 2010) is a mixed-grass prairie semiarid ecosystem dominated by C_4 grasses, C_3 grasses, forbs and sub-shrubs, and 10 samples/plots (5 each from aCO_2 and eCO_2) were taken in July 2008 when this site was exposed to eCO_2 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_2 (aCO_2) and eCO_2 (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_3-N and NH_4-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 nm/280 nm > 1.70, and 260 nm/230 nm > 1.8), more gel purification was performed. The purified DNA was stored at −80 °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 μg of amplified DNA was labeled and then hybridized with GeoChip 3.0 at 45 °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 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 eCO_2 was first standardized by aCO_2 values (e.g., 100%) for each site, and then the 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 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 aCO_2 and 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 aCO_2 data (the mean value) as 100% to standardize its corresponding 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 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 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 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 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: $s_i = \frac{x_i - Mean(x_i)}{SD(x_i)}$, where s_i is the standardized value, x_i is the original value, and Mean(x_i) and 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 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 (NO_3^-), ammonium (NH_4^+), total nitrogen (TN), total carbon (TC) and C:N ratio differed significantly by site ($p < 0.001$), but not by CO_2 treatment ($p > 0.05$), and that a site by CO_2 interaction

was only significant ($p < 0.001$) for soil NO_3^- (Fig. S3). Also, we analyzed the effects of 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 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 (NO_3-N) significantly (95% CI) decreased (Fig. 1F), while TC (Fig. 1D), TN (Fig. 1E), NH_4-N (Fig. 1G) or C:N ratio (Fig. 1H)) did not significantly change under 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 eCO_2-induced changes are expected to influence soil microbial communities and their functional potential.

3.2. Overview of soil microbial community responses to eCO_2

Based on all genes ($N = 10{,}259$) detected by GeoChip 3.0, 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 CO_2 treatment (Fig. 2A). Also, within each site, dissimilarity tests (PERMANOVA, MRPP and ANOSIM) indicated a significant ($p < 0.05$) 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, 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 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 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 eCO_2. Specifically, among 42 functional gene families involved in C, N, S and P cycling examined, common responses to 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 eCO_2, and such ecosystem functional processes, including

N_2 fixation, C decomposition, greenhouse gas emission and others. For example, four key genes involved in C fixation (*pcc/acc*), N_2 fixation (*nifH*), and labile C degradation (*amyA* and *pulA*) were significantly (95% CI) stimulated by eCO_2, and especially, the increase of *nifH* abundance at 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 eCO_2 might also increase recalcitrant C degradation potential, especially in some ecosystems like C_4 plantation ecosystems (Fig. 3B). Additionally, to obtain mechanistic insights into greenhouse gas emissions under eCO_2, we analyzed the functional gene markers *mcrA* for CH_4 generation, *pmoA* for CH_4 oxidation, *norB* for N_2O generation, and *nosZ* for N_2O reduction, and only found that *mcrA* increased significantly (at 95% CI) as a common response to eCO_2, indicating that eCO_2 might potentially increase CH_4 emissions, but might not significantly affect N_2O 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 CH_4 emissions could generally increase under 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 eCO_2, we measured ecosystem processes and

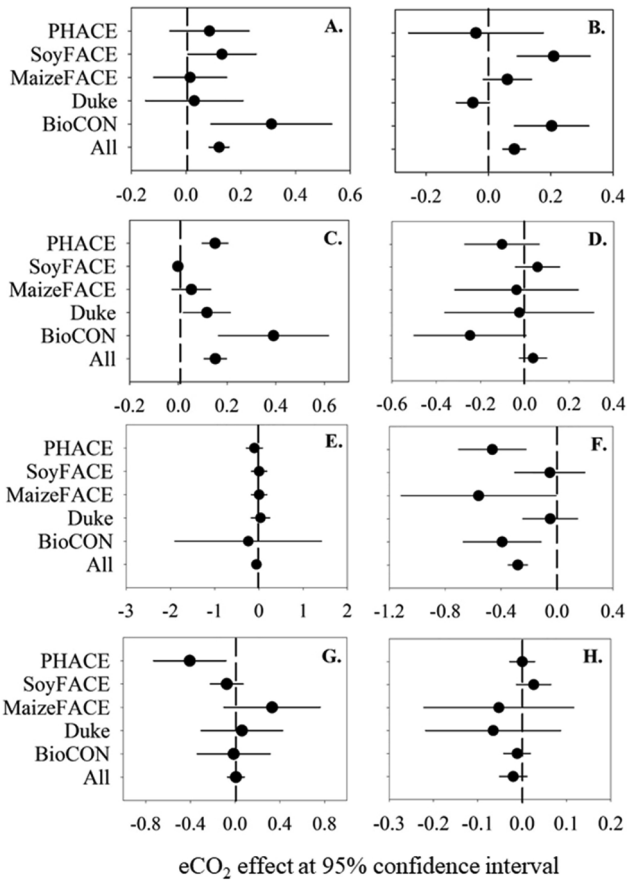

Fig. 1. Response ratio analysis of the effect of eCO_2 on soil properties and ecosystem functions at the confidence interval (CI) of 95%. (A) Biomass or net primary production (NPP); (B) Soil moisture; (C) Soil C decomposition; (D) Total soil C; (E). Total soil N; (F) Soil nitrate; (G) Soil ammonium; and H. C:N ratio. If a 95% CI did not overlap with the zero-line, the response was considered significant with a positive eCO_2 effect on the right or a negative eCO_2 effect on the left. Details for defining common and specific responses to eCO_2 and response ratio analysis of within-a-site and across-all-site samples are described in Fig. S2.

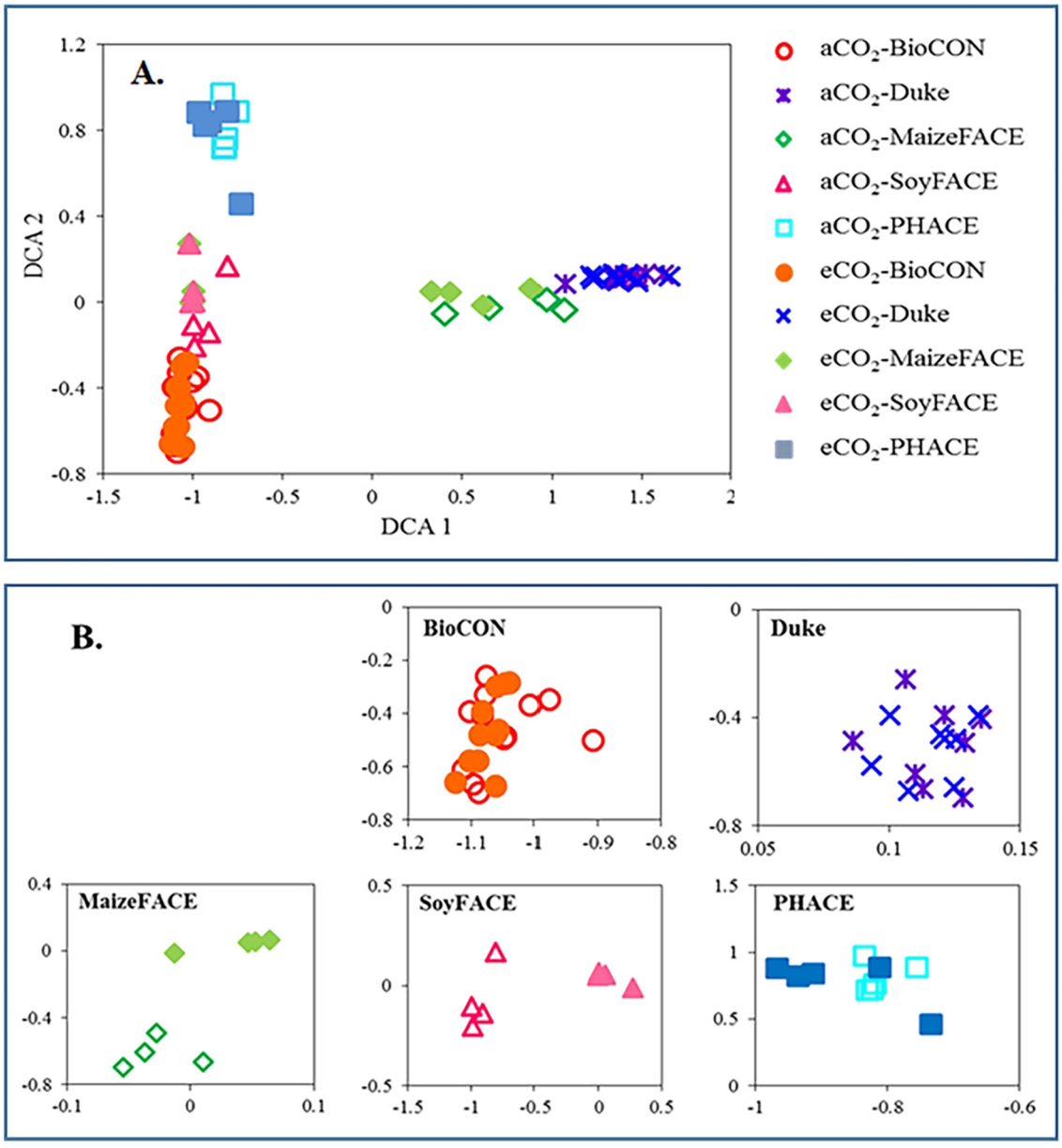

Fig. 2. Detrended correspondence analysis (DCA) of all genes (N = 10,259) detected by GeoChip 3.0 across all five disparate sites (A) and within each individual site (B), including BioCON, Duke, MaizeFACE, SoyFACE, and PHACE.

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 NO_3-N, but there was no significant changes in total soil C, N, NH_4-N or C:N ratio under 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 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%), CH_4 (~43% in rice fields) and N_2O (~19%) emissions significantly at a 95% CI (Fig. 4). Specifically, when we mapped those commonly responsive functional genes to their responsible ecosystem functions under 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 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 eCO_2. Therefore, the abundance of commonly responsive functional genes appeared to be directionally related with ecosystem functions under eCO_2.

4. Discussion

Reliable prediction of 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_2 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_2 effect (% change)[a] with response ratio testing of significance					
		All	BioCON	Duke	MaizeFACE	SoyFACE	PHACE
Acetogenesis or C fixation	*fhs*	46.4*	25.7	−11.5	**85.2****	96.3	26.6
	CODH	30.6	41.7	0.7	62.1	33.7	12.9
	pcc/acc	**54.0****	51.3**	21.8**	91.4**	64.1**	29.4
	rbcL	**50.9****	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
	amyA	**79.2****	53.9**	16.4	87.8**	95.5**	58.7**
	Glucoamylase	40.7*	17.7	7.9	**96.44****	67.9	12.0
	Endoglucanase	43.9	**106.6****	3.6	23.9	−26.4	52.7
	Exoglucanase	13.3	−35.0	**36.8****	62.8*	12.3	−11.8
	ara	27.5*	**64.3****	−5.1	17.4	9.4	33.9
	ara-fungi	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*	**68.2****
	NAG[b]	29.8*	21.8	−11.5	58.6*	48.7	23.8
	xylA	37.3	−3.9	−8.1	**99.2****	−5.9	**51.3****
	Xylanase	9.9	−11.0	2.0	38.7	23.8	−4.4
	limEH	11.7	−0.1	−19.8	−4.0	17.9	39.1
	pulA	**104.7****	46.7**	10.4	160.4**	118.1**	65.3**
	vanA	18.8	3.6	8.5	46.1	−3.9	28.4
	vdh	**78.9****	33.1	23.0	191.6**	15.6	56.8**
Recalcitrant C degradation	*lcc*	**44.6****	25.9	14.5	85.4**	58.2*	28.2
	glx	25.3	−18.9	−13.4	**112.9****	27.5	15.4
	lip	92.6	30.9	−17.9	**452.9****	13.5	−19.4
	mnp	**84.7****	36.2	128.6**	79.6	79.4**	49.9**
Methane metabolism	*mcrA*	**69.1****	25.0	15.1	108.4**	111.0	46.3*
	pmoA	40.4*	15.0	16.3	31.8	48.3	47.6*
N cycling	*nifH*	**54.9****	52.3**	14.4*	69.5**	68.4**	41.1**
	amoA	10.3	7.5	10.0	31.5	−18.2	17.3
	nasA	**121.7****	71.5**	21.0	165.3**	66.3**	73.9**
	nrfA	57.0*	**48.0****	9.4	**135.3****	41.3	33.7*
	gdh	41.4	−6.3	0.8	−41.9	**134.0****	**54.7****
	ureC	26.6	17.3	−4.2	72.2	4.8	30.0
	narG	**68.3****	32.4	6.8	128.7**	30.4	58.8**
	nirK	45.9*	45.8	5.1	68.8*	35.7	42.5
	nirS	39.9	19.3	11.4	**134.9****	13.4	17.0
	norB	8.9	4.4	4.2	38.0	−1.8	−0.3
	nosZ	27.5	6.8	−20.6	77.4	28.8	31.1
P cycling	*ppk*	23.2	3.0	8.7	**87.6****	−4.5	17.6
	ppx	17.6	7.2	−2.8	68.9*	16.8	−2.2
S cycling	*dsrA*	20.2	5.8	5.8	47.8	22.1	16.4
	dsrB	34.8*	8.9	**44.3****	49.0	11.6	37.7
	sox	15.5	4.2	−6.7	48.6	11.9	16.4

a. CO_2 effect (% change)[a] = $(eCO_2 - aCO_2) * 100/aCO_2$, where aCO_2 and eCO_2 were the average signal intensities of genes detected at aCO_2 or eCO_2, respectively; b. NAG: acetylglucosaminidase. Response ratio: **: 95% confidence interval (CI); *: 90% CI. Details for defining common and specific responses to eCO_2 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_2, 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_2, 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_2 across disparate FACE sites, and that such common responses would be directionally linked to ecosystem functioning in the future eCO_2 environment. Several possible mechanisms may explain why there are such common patterns for soil microbial communities in response to eCO_2 across disparate sites or ecosystems. First, as a general pattern, eCO_2 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_2 (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_2. Indeed in this study, we found eCO_2 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_2, more available N would be provided to plants to relieve

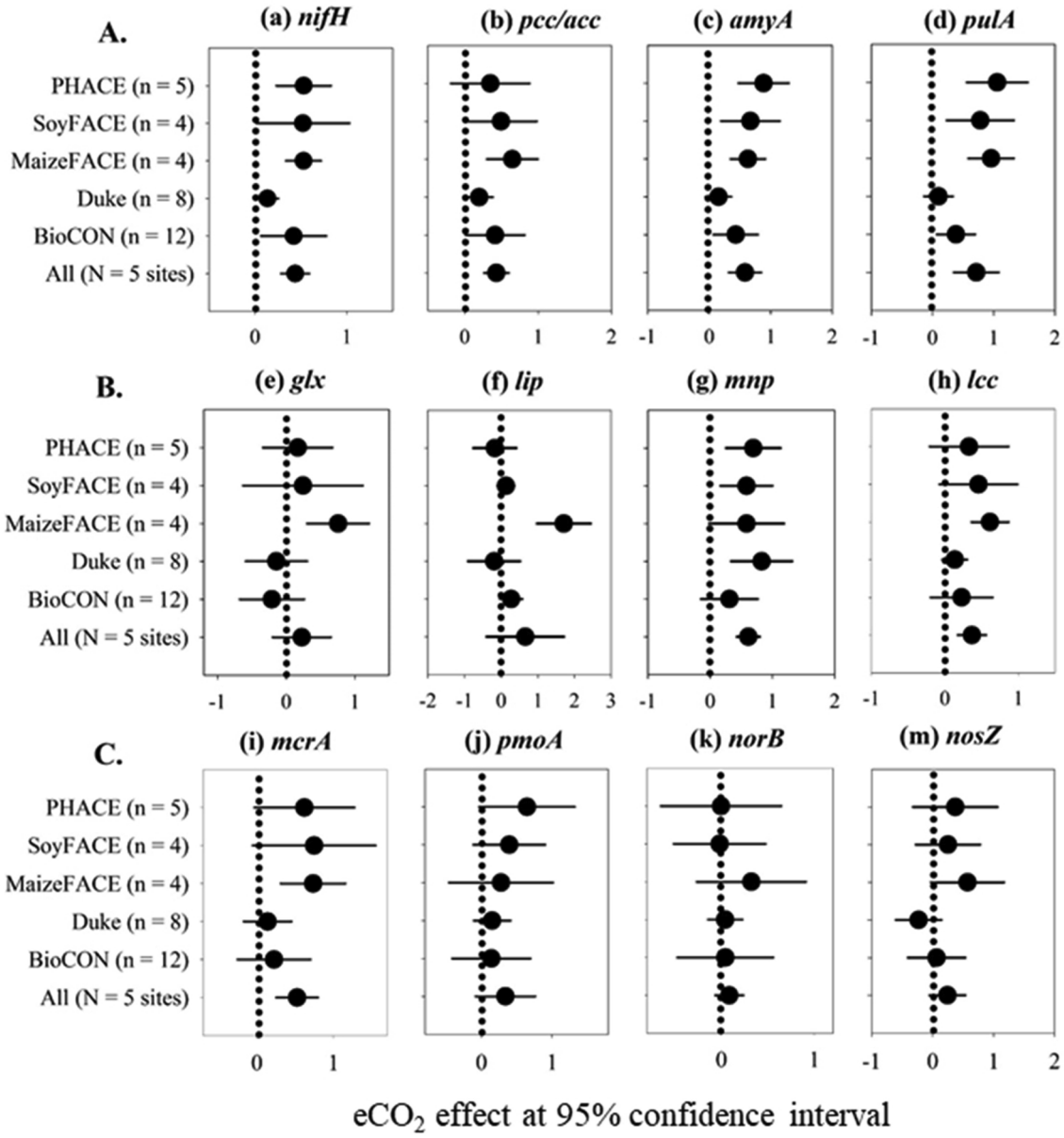

Fig. 3. Response ratio analysis of representative key functional genes in response to eCO_2 across five sites (All) and within each individual site at a 95% confidence interval (CI). (a) *pcc/acc* encoding propionyl-CoA/acetyl-CoA carboxylase for the 3-hydroxypropionate/malyl-CoA cycle (carbon fixation); (b) *nifH* encoding dinitrogenase reductase for microbial N_2 fixation; (c) *amyA* encoding α-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 eCO_2 effect on the right or a negative eCO_2 effect on the left. Details for defining common and specific responses to eCO_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 eCO_2.

Second, eCO_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 expereimental and theorical 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 eCO_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.

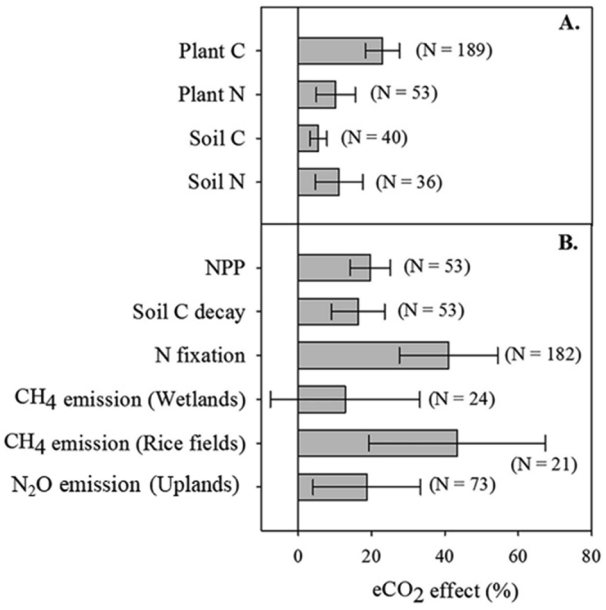

Fig. 4. Responses of terrestrial ecosystem processes to eCO_2 by response ratio analysis of published datasets across a broader range of FACE sites. The x-axis presents the eCO_2 effect (%) calculated by $\frac{(eCO2 - aCO2) * 100}{aCO2}$, where aCO_2 and eCO_2 were the average mean values at aCO_2 or eCO_2, 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_4 and N_2O 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_2. One of most significant changes in the soil environment under eCO_2 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_2 (Xu et al., 2016). For example, a previous study showed that eCO_2 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_2 due to improved soil water conditions (Rice et al., 1994). Also, soil C generally increases under eCO_2, 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_2, 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_2. 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_2 (Fig. 5). With increases in nutrient inputs, and changes in soil microenvironments, generally increased functional gene abundances were observed under eCO_2, 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_2. As N limitation appears to constrain plant responses to eCO_2 (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_2 environment (Fig. 5d). Indeed, we observed increases in N fixation and total soil N under eCO_2 in a broader range of FACE sites of the literature survey. Although this study was focused on the effect of eCO_2 on free-living N_2 fixers with bulk soil samples, previous studies also showed that eCO_2 could increase symbolic N_2 fixation and amplify the benefit of N_2 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_2 (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_2, 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_2–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_2 in our literature survey, indicating a possible C sink in the future eCO_2 environment. In addition, an increase of *mcrA* abundance at eCO_2 was observed, which appeared to be consistent with an increased trend of CH_4 emissions in rice fields in our literature survey, and a recent study, showing that eCO_2 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 it sustainability in the future eCO_2 environment (Fig. 5f). As microbial functional genes were found to be able to predict N_2O 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_2O emissions in this study, while an increase of N_2O emissions under eCO_2 was observed in upland soils (van Groenigen et al., 2011), indicating a possible lack of directional linkage between N_2O metabolic genes and N_2O 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_2 from microbial functional ecology perspectives. We identified common response patterns of microbial functional genes under eCO_2, 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

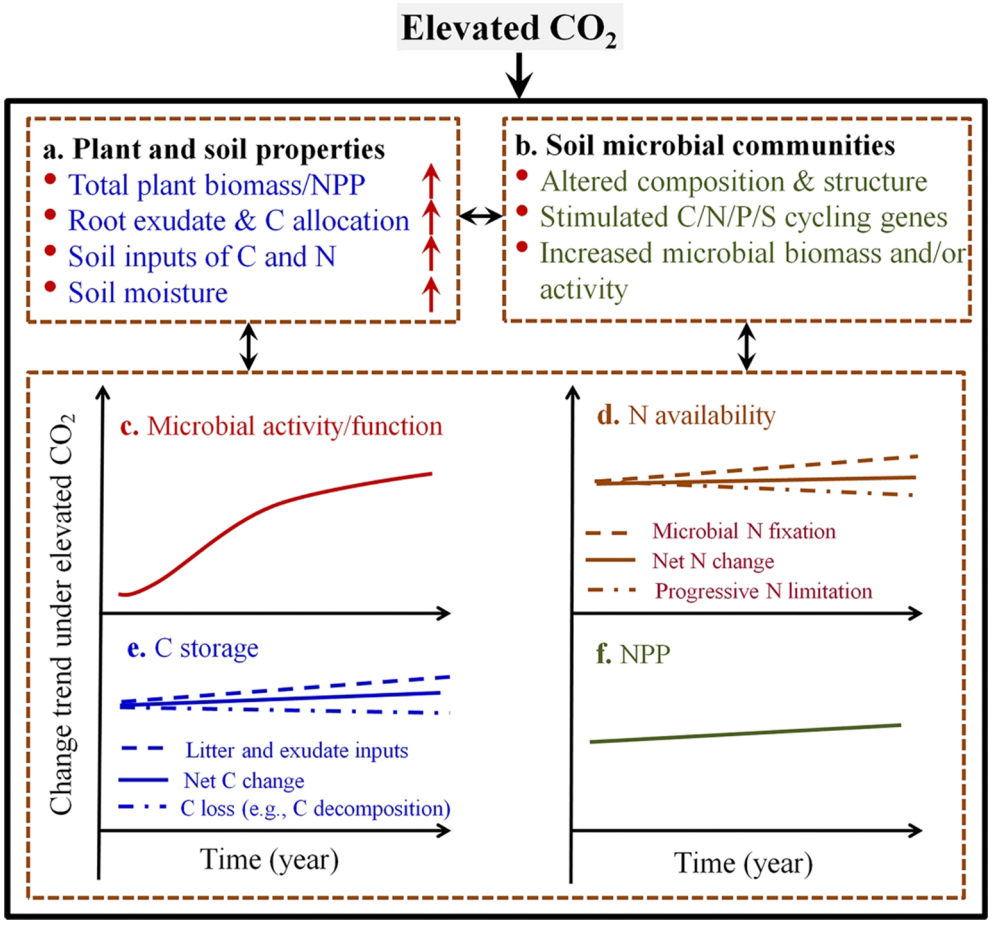

Fig. 5. A conceptual framework for linking the soil microbial community and ecosystem functioning in response to eCO_2. (a) Based on previous studies, eCO_2 generally increases plant biomass/net primary productivity (NPP) and root exudation, and alters the soil microenvironment (e.g., soil moisture), 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_2. (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_2. (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_2. (f) A stably increased NPP may be sustained in the future eCO_2 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_2, 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_2, 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_2 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.

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Microbial functional genes commonly respond to elevated carbon dioxide

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Supporting Information

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A. Description and background of five FACE experimental sites

This study was conducted at five FACE experimental sites (Fig. S1):

BioCON. The BioCON (Biodiversity, CO_2, Nitrogen deposition) experiment site is a planted native grassland containing a total of 296 plots with three treatments: CO_2 (ambient, 368 ppm vs elevated, 560 ppm), N (ambient vs. 4 g NH_4NO_3 m^{-2} $year^{-1}$), and plant diversity (1, 4, 9 or 16 native grass species in four functional groups: C_3, C_4, forb and legume (Reich et al. 2001). Previous studies showed increased soil pH, soil moisture, and bacterial biomass, and shifts of both phylogenetic and functional composition, structure and interaction network of soil microbial communities under eCO_2 (Deng et al. 2012; He et al. 2012; He et al. 2010b; Zhou et al. 2010; Zhou et al. 2011).

Duke FACE. The Duke Forest FACE experiment is a pine-dominated (>90% of basal area) forest ecosystem. Elevated CO_2 concentration is maintained 200 ppm (e.g., 585 ppm when the samples were taken for this study) above the ambient level (e.g., 385 ppm). Soils are highly weathered clay loams (mixed thermic Ultic Hapludalfs), and a detailed description of the site can be found in Lichter et al. (Lichter et al. 2008). Soil moisture tended to increase at eCO_2 (Norby et al. 2010) although soil pH varied relatively little (4.1 to 5.2) between aCO_2 and eCO_2 samples (Ge et al. 2010). Previous studies showed that limited available N in soil constrained C sequestration at eCO_2 (Norby et al. 2010), while eCO_2 increased the release of soluble C from roots to soil, thus accelerating turnover of N pools in the rhizosphere (Phillips et al. 2011). Another study showed that the acid to aldehyde ratios of lignin-derived phenols increased and leaf-derived alkyl structures were enriched under eCO_2 and N fertilization, suggesting an enhanced degradation of lignin and hydrolysable lipid components (Feng et al. 2010).

SoyFACE/MaizeFACE. The SoyFACE is a typical corn-soybean rotation agroecosystem with a randomized complete block design (n = 4) with each block containing four treatments: (i) ambient

CO_2 (~400 ppm in 2008) and O_3 (~37.9 ppb in 2008), (ii) elevated CO_2 (~550 ppm), (iii) elevated O_3 (~ 61.3 ppb in 2008), and (iv) a combination of elevated CO_2 and O_3. The soil is Drummer–Flanagan (fine-silty, mixed, mesic Typic Endoaquoll) with a pH of 5.73–6.14, which was not significantly affected by eCO_2 (Peralta and Wander 2008), but eCO_2 did generally increase soil moisture for both SoyFACE and MaizeFACE experiments (Leakey et al. 2009). It was also found that eCO_2 increased N turnover rates, but did not increase N_2O emissions at SoyFACE (Decock et al. 2012; Decock and Six 2011). It is hypothesized that legumes like soybean have a competitive advantage over non-legumious species at eCO_2 (Rogers et al. 2009), while C_4 plants (e.g., corn) may not be as sensitive as C_3 grasses or other plants in response to eCO_2 (Leakey et al. 2009). However, similar responses of soil microbial communities to eCO_2 were observed recently (Xiong et al. 2015).

PHACE. The PHACE (Prairie Heating and Carbon Dioxide Enrichment) experiment includes a factorial combination of two levels of CO_2 (ambient 400 ppm vs elevated 600 ppm) and two temperature (ambient vs elevated with 1.5/3.0^{o}C warmer day/night) regimes with five replications for each treatment randomly assigned 20 (3.3-m diameter) circular plots. The soil is a fine-loamy, mixed, mesic Aridic Argiustoll with a pH of 7.9, which was not significantly affected by eCO_2, while soil moisture significantly increased at eCO_2 (Dijkstra et al. 2010). It is a mixed-grass prairie semiarid ecosystem dominated by C_4 grasses, C_3 grasses, and forbs and sub-shrubs, and details of the experimental site, design and setup are as previously described (Dijkstra et al. 2010). A previous study showed that microbially mediated CH_4 consumption was significantly higher but N_2O emission was not significantly affected under eCO_2 (Dijkstra et al. 2010), and another study indicated that eCO_2 completely reversed the desiccation effects of moderate warming, and favored C_3 grasses and enhanced stand productivity, whereas warming favored C_4 grasses (Morgan et al. 2011). A recent laboratory incubation study from PHACE showed that eCO_2 microbial

80 communities had an increased ability to decompose soil organic matter (SOM) compared with
81 those from aCO_2 plots, suggesting positive feedbacks of soil microbial communities to this semi-
82 arid ecosystem (Nie et al. 2013).

B. Supplementary tables

Table S1 Summary information about five FACE experimental sites/ecosystems in this study.

Project	**BioCON**[a]	**Duke**[b]	**MaizeFACE**[c]	**SoyFACE**[d]	**PHACE**[e]
Site	Cedar Creek Ecosystem Science Reserve, MN	Duke Forest, NC	Urbana-Champaign, IL		Cheyenne, WY
Ecosystem	Native C_3 grass, C_4 grass, legume, and forb species	Loblolly pine forest	Corn/Soybean rotation		Mixed grass prairie
Elevated CO_2	560 ppm	585 ppm	550 ppm		600 ppm
Other treatment	Plant diversity, and nitrogen	Soil nutrients	O_3, temperature, and drought		Temperature
Lat/Lon	45˚24' N/ 93˚12' W	35˚58' N/ 79˚5' W	40˚2' N/88˚13' W		41˚11'N 104˚54'W
Start-end year	1997-	1994-2010	2001-		2006-
Ring/block/plot	6	8	8	8	2
Field replicate	12	8	4	4	5
Total plot	24	16	8	8	10
Depth	0-15 cm	0-10 cm	0-15 cm	0-15 cm	0-15 cm
Sampling time	July 2007	July 2008	October 2008	May 2009	July 2008
eCO_2 exposure	10 years	15 years	7 years	7.5 years	2 years

a. BioCON (Biodiversity, CO_2 and Nitrogen): http://www.biocon.umn.edu/
b. Duke Forest-Atmosphere Carbon Transfer and Storage (FACTS-I): http://face.env.duke.edu/main.cfm/
c/d. MaizeFACE and SoyFACE: http://soyface.illinois.edu/index.htm/
e. PHACE (Prairie Heating and CO_2 Enrichment): http://www.ars.usda.gov/Research/docs.htm?docid=16754/

Table S2 Dissimilarities of soil microbial communities between eCO_2 and aCO_2 samples across all five sites and within each individual site based on the abundance of all detected functional genes (N = 10,259) by GeoChip 3.0. Significant p-values ($p < 0.05$) are **bold** in the table. 33 samples for aCO_2 and eCO_2 each were normalized from all five sites.

Site or ecosystem	MRPP[a]		ANOSIM[b]		PERMANOVA[c]	
	Delta	p	R	p	F	p
All	0.437	**0.001**	0.754	**0.001**	10.344	**0.001**
BioCON	0.522	**0.001**	0.248	**0.002**	2.868	**0.002**
Duke	0.344	0.056	0.235	0.065	2.191	0.072
MaizeFACE	0.388	**0.033**	0.552	**0.025**	3.024	**0.004**
SoyFACE	0.433	**0.026**	0.500	**0.024**	2.678	**0.001**
PHACE	0.425	0.091	0.224	0.115	1.877	0.121

[a]MRPP: a nonparametric procedure that does not depend on assumptions such as normally distributed data or homogeneous variances, but rather depends on the internal variability of the data; [b]ANOSIM: analysis of similarity; [c]PERMANOVA: permutational multivariate analysis of variance evaluated using the adonis function implemented in the R statistical environment (vegan library).

Table S3 The effect of eCO_2 on the abundance of all functional genes detected at the whole community level (N =10,259), for C, N, P and S cycling categories, and for representatives of functional gene families (He et al. 2010a) revealed by the permutational (nonparametric) multivariate analysis of variance (PERMANOVA) with the *adonis* function.

Whole/category/gene	Bray-Curtis[a]		Jaccard[b]	
	F value	P value	F value	P value
Whole community	0.736	0.116	1.344	**0.001**
Functional category				
C cycling	0.744	0.065	1.349	**0.001**
N cycling	0.734	0.102	1.414	**0.001**
P cycling	0.714	0.192	1.755	**0.001**
S cycling	0.757	0.101	1.369	**0.001**
Functional gene family				
amyA	0.872	**0.046**	1.631	**0.001**
pulA	1.179	**0.006**	1.714	**0.001**
glx	0.687	0.206	0.883	**0.032**
lip	0.679	0.509	1.458	**0.040**
mnp	1.474	0.077	1.817	**0.005**
mcrA	0.751	0.203	1.304	**0.002**
pmoA	0.739	0.325	1.327	**0.005**
amoA	0.585	0.520	0.905	0.084
nifH	0.593	0.298	1.227	**0.001**
nirK	0.615	0.479	0.895	**0.021**
nirS	1.061	**0.030**	2.124	**0.001**
norB	0.770	0.309	1.101	**0.015**
nosZ	1.195	**0.021**	2.087	**0.001**
gdh	1.050	0.116	1.088	0.115
ureC	0.838	0.136	1.437	**0.001**

a: the distance was calculated by the Bray-Curtis method (quantitative); b: the distance was calculated by the Jaccard method (non-quantitative).

Table S4 Correlations between functional gene categories or families and soil properties analyzed by Mantel tests. Significant *p*-values ($p < 0.05$) are **bold**. All includes soil properties of total C (TC), total nitrogen (TN), NO_3^-, NH_4^+, and the ratio of TC to TN (C:N). Details about those functional genes are described previously (He et al. 2010a).

Gene category	*rM/p*	All	TC	TN	NO^{3-}	NH^{4+}	C:N
All genes detected	*rM*	0.308	0.268	-0.064	-0.092	0.247	0.504
	p	**0.001**	**0.003**	0.849	0.899	**0.001**	**0.001**
Carbon cycling	*rM*	0.343	0.296	-0.062	-0.104	0.287	0.554
	p	**0.001**	**0.001**	0.871	0.946	**0.001**	**0.001**
Energy process	*rM*	0.252	0.183	-0.039	-0.019	0.172	0.395
	p	**0.004**	**0.009**	0.724	0.576	**0.025**	**0.001**
Metal reduction or resistance	*rM*	0.265	0.236	-0.057	-0.079	0.203	0.444
	p	**0.001**	**0.004**	0.827	0.824	**0.018**	**0.001**
Nitrogen cycling	*rM*	0.318	0.253	-0.073	-0.050	0.253	0.509
	p	**0.001**	**0.001**	0.902	0.729	**0.005**	**0.001**
Organic remediation	*rM*	0.239	0.220	-0.044	-0.095	0.184	0.403
	p	**0.002**	**0.011**	0.770	0.893	**0.013**	**0.001**
Others (e.g., *gyrB*)	*rM*	0.389	0.350	-0.083	-0.068	0.268	0.663
	p	**0.001**	**0.001**	0.927	0.825	**0.002**	**0.001**
Phosphorus cycling	*rM*	0.303	0.174	-0.057	0.069	0.197	0.430
	p	**0.001**	**0.001**	0.841	0.177	**0.003**	**0.001**
Sulfur cycling	*rM*	0.351	0.308	-0.094	-0.111	0.305	0.566
	p	**0.001**	**0.001**	0.952	0.944	**0.001**	**0.001**
Functional gene family/enzyme							
amyA	*rM*	0.288	0.305	-0.059	-0.140	0.190	0.513
	p	**0.002**	**0.001**	0.833	0.987	**0.018**	**0.001**
ara (bacteria)	*rM*	0.278	0.174	-0.074	-0.056	0.338	0.461
	p	**0.001**	**0.015**	0.894	0.741	**0.001**	**0.001**
ara (fungi)	*rM*	0.354	0.287	-0.094	-0.033	0.303	0.562
	p	**0.001**	**0.002**	0.971	0.621	**0.001**	**0.001**
Cellobiase	*rM*	0.385	0.367	0.011	-0.116	0.273	0.555
	p	**0.001**	**0.002**	0.383	0.971	**0.004**	**0.001**
Endo-chitinase	*rM*	0.415	0.346	-0.042	-0.005	0.243	0.611
	p	**0.001**	**0.001**	0.720	0.465	**0.010**	**0.001**
Endo-glucanase	*rM*	0.319	0.309	-0.039	-0.122	0.250	0.538
	p	**0.001**	**0.001**	0.741	0.966	**0.007**	**0.001**
Exo-glucanase	*rM*	0.284	0.102	-0.043	-0.027	0.360	0.322
	p	**0.002**	0.098	0.759	0.569	**0.002**	**0.004**
Acetylglucosaminidase	*rM*	0.204	0.145	-0.118	-0.027	0.201	0.365
	p	**0.012**	0.058	0.989	0.572	**0.014**	**0.001**
Xylanase	*rM*	0.402	0.355	-0.089	-0.056	0.287	0.633
	p	**0.001**	**0.001**	0.929	0.741	**0.002**	**0.001**
lip	*rM*	-0.056	0.008	0.036	-0.113	-0.067	-0.029
	p	0.766	0.387	0.239	0.992	0.835	0.582
CODH	*rM*	0.388	0.366	0.005	-0.118	0.301	0.554
	p	**0.001**	**0.001**	0.431	0.955	**0.003**	**0.001**
nasA	*rM*	0.232	0.172	-0.114	-0.072	0.228	0.479
	p	**0.004**	**0.014**	0.992	0.823	**0.005**	**0.001**

nirK	*rM*	0.145	0.131	-0.012	0.023	0.082	0.179
	p	**0.043**	0.076	0.558	0.319	0.210	**0.037**
*nir*S	*rM*	0.289	0.226	-0.056	-0.022	0.249	0.390
	p	**0.003**	**0.020**	0.810	0.555	**0.008**	**0.001**
nifH	*rM*	0.385	0.306	-0.052	-0.049	0.267	0.609
	p	**0.001**	**0.001**	0.800	0.752	**0.003**	**0.001**
ppk	*rM*	0.311	0.240	-0.119	-0.131	0.289	0.510
	p	**0.001**	**0.003**	0.993	0.976	**0.002**	**0.001**
ppx	*rM*	0.279	0.131	-0.027	-0.050	0.147	0.361
	p	**0.001**	**0.047**	0.681	0.710	**0.042**	**0.001**
sox	*rM*	0.227	0.191	-0.043	0.121	0.300	0.305
	p	**0.004**	**0.016**	0.729	0.064	**0.001**	**0.001**
dsrA	*rM*	0.376	0.342	-0.077	-0.070	0.265	0.589
	p	**0.001**	**0.001**	0.918	0.838	**0.003**	**0.001**

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C. Supplementary figures

Figure S1. Locations (★) of five FACE experimental sites of USA in this study. They are BioCON, Duke, MaizeFACE, SoyFACE, and PHACE. Details about those sites are described in the Supplementary Information A (Description and background of five FACE experimental sites) and Table S1. Geographic distances range from meters within a site of different plots to a maximum of 2302 km between the Duke Forest site and the PHACE site.

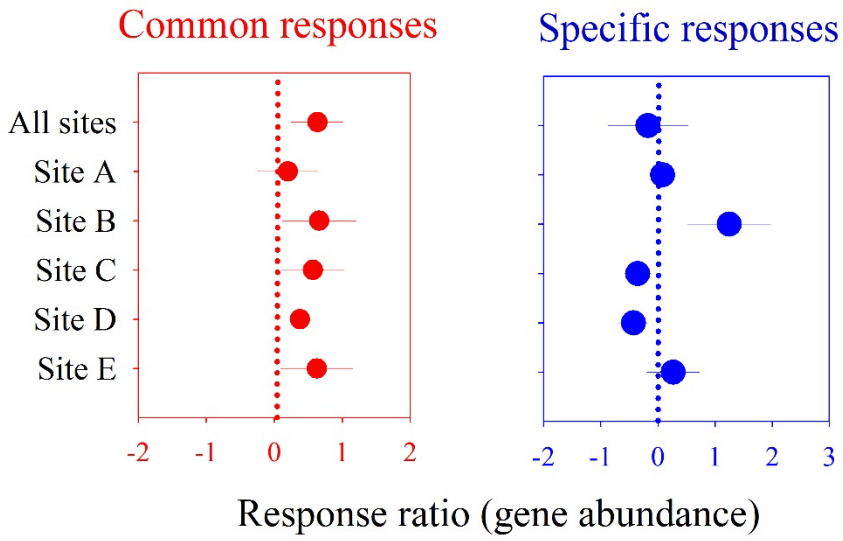

Figure S2. A schematic presentation used to define common and specific responses of soil properties, ecosystem processes, and microbial functional genes to eCO_2 using response ratio analysis. In this study, we used response ratio analysis at two levels: (i) within-a-site and (ii) across-all-sites. For within-a-site analysis, the original data were used for both aCO_2 and 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 aCO_2 data (the mean value) as 100% to standardize its corresponding 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 across-all-site results, a common response is defined as a significant (e.g., 95% confidence interval) change of functional gene abundance across all sites under eCO_2 although such a significant change may not be seen within each individual site (Site A, B, C, D, E or F), while a specific response is defined as non-significant change across all sites under eCO_2, but such a change may be significant (e.g., 95% confidence interval) generally within one or two sites. Common responses may be used to predict ecosystem functioning and stability at a global/regional scale.

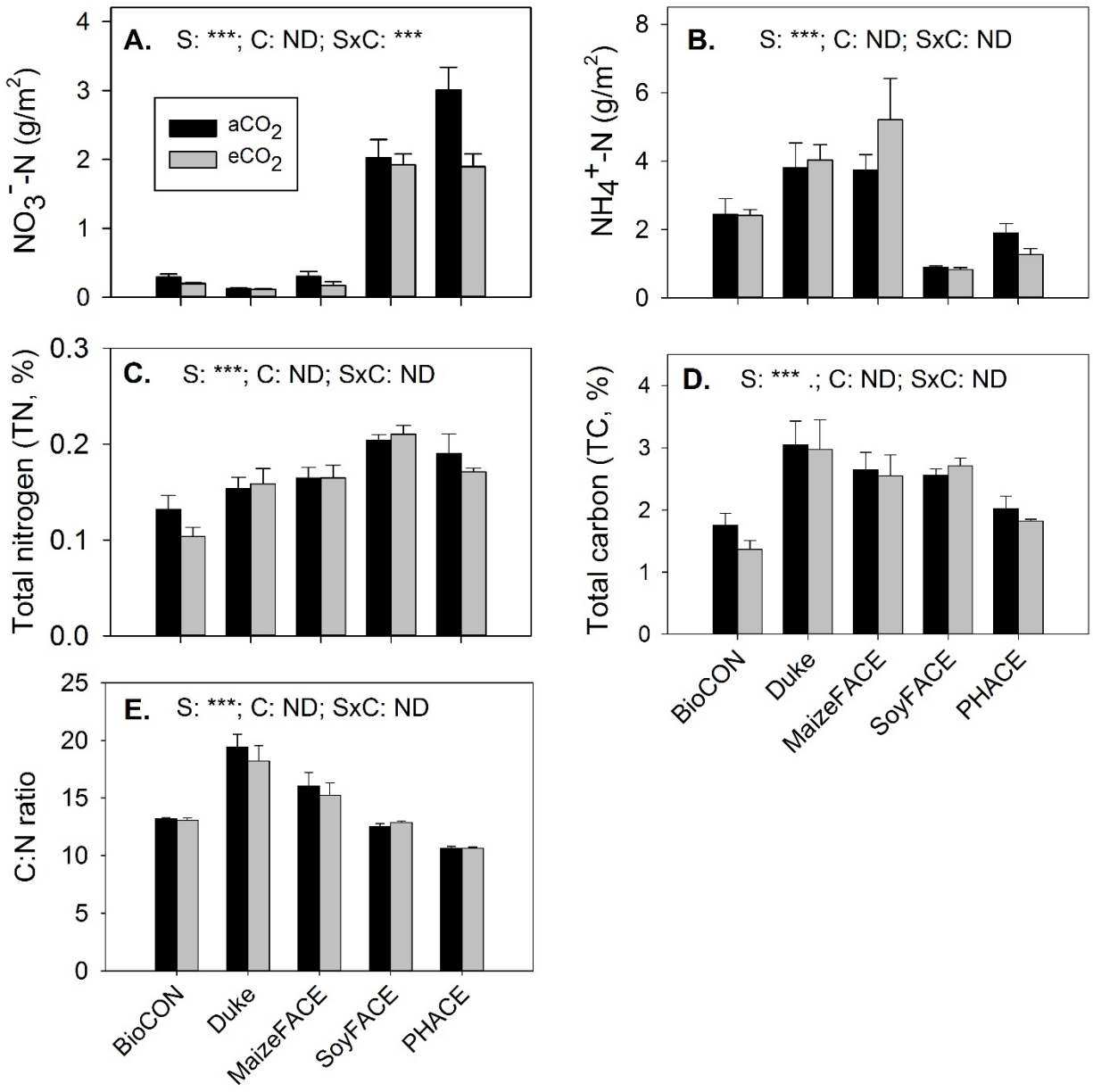

Figure S3. The effect of site, CO_2 and their combination on soil properties analyzed by ANOVA. The ANOVA results are showed as: S: site; C: CO_2; SxC: site x CO_2 across all five ecosystems, and their significances are presented as: ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; ND: no difference ($p > 0.05$). A. NO_3^- (g/m^2); B. NH_4^+ (g/m^2); C. total nitrogen (TN, %); D. total carbon (TC, %), and E. C:N ratio. Data are presented as mean ± standard error (error bars).

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