Elevated carbon dioxide accelerates the spatial turnover of soil microbial communities

Journal:	*Global Change Biology*
Manuscript ID:	Draft
Wiley - Manuscript type:	Primary Research Articles
Date Submitted by the Author:	n/a
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Keywords:	elevated carbon dioxide, spatial turnover rate, microbial community, free air CO2 enrichment , β-diversity , CO2 increases
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Elevated carbon dioxide accelerates the spatial turnover of soil microbial communities

Running head: eCO_2 accelerates microbial spatial turnover

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Paper type: Primary Research Article

Abstract

Although elevated CO_2 (eCO_2) significantly affects the α-diversity, composition, function, interaction and dynamics of soil microbial communities at the local scale, little is known about its impacts on the geographic distribution of microorganisms regionally or globally. Here we examined the β-diversity of 110 soil microbial communities across six free air CO_2 enrichment (FACE) experimental sites using a high-throughput functional gene array. The β-diversity of soil microbial communities was significantly ($p < 0.05$) correlated with geographic distance under both CO_2 conditions, but declined significantly ($p < 0.05$) faster at eCO_2 (-0.0250) than at ambient CO_2 (aCO_2, -0.0231) though it varied within each individual site, indicating that the spatial turnover rate of soil microbial communities was accelerated under eCO_2 at a large geographic scale (e.g., regionally). Both distance and soil properties significantly ($p < 0.05$) contributed to the observed microbial β-diversity. This study provides new hypotheses for further understanding their assembly mechanisms, especially as threat from global CO_2 increases.

Introduction

Many lines of evidence show that elevated CO_2 (eCO_2) generally shifts the composition, structure, and interaction of soil microbial communities and their ecosystem functioning (Blagodatskaya *et al.*, 2010, Carney *et al.*, 2007, Deng *et al.*, 2012, He *et al.*, 2012a, He *et al.*, 2010b, van Groenigen *et al.*, 2014, Zhou *et al.*, 2011). However, most of those studies were conducted within individual sites, and it remains unclear how eCO_2 affects the geographic distribution (e.g., distance-decay relationship) of soil microorganisms and their associated ecological processes. The distance-decay relationship has been widely used to understand geographic patterns of biodiversity and community assembly mechanisms across a range of organisms and environmental gradients over a temporal and spatial scale (Hanson *et al.*, 2012). Two ecological theories have been proposed to explain the distance-decay relationship in both macrobiology and microbiology. One is the niche assembly theory, which predicts the biodiversity of a community is maintained by partitioning of organisms to specialized niches so that certain number of species can coexist in a close proximity (Webb *et al.*, 2002), and the other is the neutral theory, which asserts that a community's history of stochastic dispersal and random events (e.g., extinction, speciation) is largely responsible for biodiversity patterns in nature (Hubbell, 2001). Both theories have gained support from previous studies in different ecosystems (Fierer & Jackson, 2006, Finkel *et al.*, 2012, Hanson *et al.*, 2012, Horner-Devine *et al.*, 2004, Martiny *et al.*, 2011a, Zhou *et al.*, 2014, Zhou *et al.*, 2008). Therefore, it is important to understand the distance-decay relationship of soil microbial communities and their assembly mechanisms for the maintenance of biodiversity in response to long-term eCO_2 exposure across disparate ecosystems.

Four processes (selection, drift, dispersal, and mutation) have been proposed to create and maintain microbial biogeographic patterns on inseparable ecological and evolutionary scales (Hanson *et al.*, 2012). The detection of those ecological processes for maintaining biodiversity and supporting ecosystem functions is expected to be more sensitive with higher resolution markers (Horner-Devine *et al.*, 2004). For example, the 16S rRNA gene (largely at the genus/sub-family level) may not be ideal for detecting drift or mutation (Hanson *et al.*, 2012), while functional genes (at a resolution of species/strain level), such as *amoA* and *nifH* may be better molecular markers for such purposes (Martiny *et al.*, 2011a, Zhou *et al.*, 2008). Therefore, to understand the assembly mechanisms of soil microbial communities, it is necessary to comprehensively survey the distance-decay relationship with various key functional genes.

In this study, we hypothesized that the similarity of soil microbial communities would decline as distance increased, and the turnover rate would be higher at eCO_2 than at aCO_2 largely due to increased soil carbon (C) inputs and altered microenvironments (van Groenigen *et al.*, 2014). To test those hypotheses, we analyzed the functional β-diversity of 110 soil microbial community samples (with 55 each from aCO_2 and eCO_2) from six FACE experimental sites (BioCON, Duke, ORNL, MaizeFACE, SoyFACE and PHACE) in a distance range of < 1 m to > 2300 km using a comprehension functional genes array, GeoChip 3.0 (He *et al.*, 2010a). Our results indicated that the spatial turnover rate of soil microbial communities was accelerated under eCO_2 across such a distance range.

Methods and Materials

A total of 110 soil samples were taken from six FACE experimental sites across United States (Figure S1) with 55 each from aCO_2 and eCO_2 plots. Details about sampling sites were described in the Supplemental Materials and Methods. Within each site, five to twelve replicate samples were taken under each CO_2 condition. Since the distribution of the sampling plots and their sizes were different in six sites, the distance among replicate samples within each site varied from 2.5 to 864 meters. Soil DNA was extracted from each sample, and the functional gene microarray, GeoChip 3.0 was used to analyze key functional genes involved in important ecological processes, and details of target preparation, labeling and microarray hybridization as well as data analysis are described previously (He *et al.*, 2012b, He *et al.*, 2010b). The GeoChip 3.0 contains about 28,000 probes from 292 functional gene families involved in carbon, nitrogen, phosphate and sulfate cycling, energy metabolism, metal resistance and organic contaminant degradation (He *et al.*, 2010a). Most functional gene families have specific probes derived from 100 to 2000 species/genera, thus the GeoChip could be considered as a specific, sensitive and quantitative tool to detect multiple subsets of microorganisms with certain ecological functions. Also, the phylogenetic marker, *gyrB* gene was integrated into this GeoChip as it could be used to detect specific microorganisms. Soil properties, such as NO_3-N, NH_4-N, total C and total nitrogen (N) were measured across all experiment sites as previously described (He *et al.*, 2010b).

The β-diversity of soil microbial communities was measured by the Sørensen method. The distance-decay relationship was plotted as logarithmic similarity against logarithmic distance. A least square of linear regression was used to obtain the slope. To examine the significance of distance-decay relationships, we tested if those slopes were significantly less than zero (Martiny *et al.*, 2011b) by permutated 1000 times. Also, the obtained standard deviations of

slopes from permutations of aCO_2 and eCO_2 were used to test whether they are significantly different. It was considered as significant if $p < 0.05$ in this study. The statistical analyses, such as permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) and multiple regression on matrices (MRM) (Legendre *et al.*, 1994) were implemented to disclose the relationships among geographic distances, environmental and microbial dissimilarities.

Results and Discussions

Analysis of soil properties in the six FACE sites by ANOVA showed that soil nitrate, ammonium, total nitrogen (TN), total carbon (TC) and C:N ratio significantly differed by site ($p < 0.001$), but not by CO_2 ($p > 0.05$), and that their interaction was only significant ($p < 0.001$) for soil nitrate (Table S4). Under eCO_2, previous studies showed that soil moisture was increased in five of six sites except ORNL, and that soil pH was increased in BioCON but remained unchanged in other five sites (Supplementary Information A).

By using GeoChip hybridization signals from 110 samples, the distance-decay rate of soil microbial communities was calculated as the slope of a linear regression on the relationship between geographic distance and community similarity under aCO_2, eCO_2, or both conditions. Although the distance-decay rates varied within individual sites (Figure S2), they were significant with the slopes less than zero: -0.0231 ($r = -0.250$, $p < 0.001$) for aCO_2 and -0.0250 ($r = -0.319$, $p < 0.001$) for eCO_2 (Figure 1A) at the overall scale (pairwise against each other). Permutation tests indicated that those two slopes were significantly ($p < 0.001$) different. When the distance-decay rates were calculated only across six sites (without consideration of those rates within each site), steeper slopes were observed for both eCO_2 (slope = -0.337, $r = -0.431$, $p < 0.001$) and aCO_2 (slope = -0.290, $r = -0.338$, $p < 0.001$), and the slope of eCO_2 was significantly ($p < 0.001$) steeper than that of aCO_2. The results suggested a higher distance-decay rate of soil microbial communities under eCO_2 compared to aCO_2 condition across six experimental sites.

Also, a partial multiple regression on matrices (MRM) (Martiny *et al.*, 2011a) further identified the relative importance of distance and soil properties contributing to such distance-decay relationships. For the overall MRM model with all variables (distance, total C, total N,

nitrate, ammonium, and C:N ratio) selected, they were significant ($p = 0.001$) with proportions (R^2): 0.237 for both aCO_2 and eCO_2, 0.228 for aCO_2, and 0.284 for eCO_2 microbial community similarities (Table 1). For individual properties, soil C:N ratio made the largest contribution with partial regression coefficients of 0.179~0.219 ($p = 0.001$), followed by soil ammonium (0.117~0.138, $p = 0.001$), and distance (0.014~0.018, $p = 0.001$). Total C, nitrate, and total N were not significant ($p > 0.05$) for aCO_2 only, eCO_2 only, or for either aCO_2, eCO_2, or both, respectively (Table 1). The results indicated that C:N ratio and ammonium were identified as major drivers of the β-diversity of soil microbial communities at eCO_2.

We further analyzed the distance-decay relationship for key functional genes/categories with more than 200 probes, which allowed more robust and reliable detection of specific functional populations. The results showed significant distance-decay relationships at the functional category and gene levels under aCO_2 or eCO_2 (Table S4). Furthermore, most of those functional genes/groups (e.g., *amyA*, phenol oxidase, endochitinase, *nifH*, *nirS*, *nirK*, *norZ*, *dsrA*, *ppx*) had steeper slopes at eCO_2 than at aCO_2, and especially the phylogenetic marker, *gyrB* also showed the same trend (Table S5). The results suggest that eCO_2 also accelerated the distance-decay rate of functional sub-communities at the functional category and/or gene level.

Understanding the mechanisms that generate and maintain biodiversity is the key to predicting the response of ecosystems to future global change. In this study, we found that the turnover rate of soil microbial communities was higher under eCO_2 at a spatial scale of 2.5 m to 2300 km, and that soil C:N ratio and ammonium could largely contribute to this observation. Our results provide new hypotheses for further understanding their assembly mechanisms.

It was hypothesized that eCO_2 would accelerate the decline of functional β-diversity of soil microbial communities with geographic distance, and the changes of distance-decay

relationship would be largely driven by environmental variation. Consistent with those hypotheses, our results are well explained by the niche assembly theory (Webb *et al.*, 2002). As Hanson et al. (Hanson *et al.*, 2012) proposed that four ecological and/or evolutionary processes: selection, drift, dispersal, and mutation would shape the microbial biogeography. In this study, our results suggest environmental selection may play a major role for structuring those microbial communities by MRM analysis, showing that soil properties except total N had larger regression coefficients than distance.

Why was a higher distance-decay rate observed at eCO_2? There are several possibilities. First, soil C inputs increase at eCO_2 (van Groenigen *et al.*, 2014), and this may drive microorganisms from oligotrophy-dominant to copiotroph-dominant communities, resulting in microbial composition changes. Indeed, we found that total soil C significantly contributed to the distance-decay relationship under eCO_2 but not under aCO_2. Second, soil N availability tends to decrease as progressive N limitation generally occurs at eCO_2 (Reich & Hobbie, 2013). An increased abundance of N_2-fixing communities at eCO_2 was identified as a common pattern across disparate sites (He et al., 2015, unpublished), possibly leading to altered N transformation soil microbial communities at eCO_2. Also, a recent study showed that microbial spatial turnover rates (z values) increased under long-term inorganic fertilization in grassland soils (Liang *et al.*, 2015). Indeed, this study found that nitrate was a significant contributor to the distance-decay relationship under aCO_2 but not under eCO_2 though C:N ratio and ammonium were found to be most important contributors to this relationship under both CO_2 conditions. Third, soil moisture generally increases at eCO_2, which may stimulate microbial activity, especially microorganisms involved in C decomposition and N cycling (He *et al.*, 2010b, van Groenigen *et al.*, 2014), further driving the convergence of soil microbial communities under eCO_2. Therefore, all those

possible reasons point to possible mechanisms shaping soil microbial communities, such as election and/or drift for generating the distance-decay relationship and increase its spatial turnover rate under eCO_2 across those six sites. Dispersal and mutation may also increase at eCO_2, but they may not significantly impact the distance-decay relationship across those six sites.

However, the distance-decay relationship varied within individual sites, and this appears to be contradictory with a previous study of ammonium-oxidizing bacterial communities within salt marsh sediments, which showed a significant distance-decay relationship (Martiny *et al.*, 2011a). Possible reasons may include a narrow distance scale, limited number of experimental plots for each site, and/or intertwining of multiple processes. For example, dispersal may entirely counteract compositional differentiation imposed by draft and selection, eliminating the distance-decay relationship (Hanson *et al.*, 2012).

Some of microbial biogeography studies were performed in environmentally uniform or controlled systems (Bell, 2010, Martiny *et al.*, 2011a), which is expected to better reveal the distance-decay relationship and understand community assembly mechanisms. However, it is very difficult to find same or similar ecosystems or environments in nature if possible. As a result, some studies of the distance-decay relationship of microbial communities have been conducted among disparate ecosystems or environments (Knief *et al.*, 2010, Ranjard *et al.*, 2013). Therefore, it is necessary to comprehensively survey the distance-decay relationship and understand their assembly mechanisms among disparate ecosystems and environments.

In summary, this study showed that eCO_2 accelerated the distance-decay relationship of soil microbial communities across disparate sites, which may be largely due to environmental selection and/or drift, providing new hypotheses for further understanding their assembly

mechanisms. Our results imply that eCO_2 may affect geographic patterns of soil microbial communities at the future eCO_2 environment.

Acknowledgements

This work was supported by the US Department of Agriculture (Project 2007-35319-18305) through the NSF-USDA Microbial Observatories Program, by the US Department of Energy, Biological Systems Research on the Role of Microbial Communities in Carbon Cycling Program (DE-SC0004601) as well as by the National Science Foundation under Grant Numbers DEB-0716587 and DEB-0620652 as well as the DEB-0322057, DEB-0080382, DEB-0218039 DEB-0219104, DEB-0217631, DEB-0716587 BioComplexity, LTER and LTREB projects, the DOE Program for Ecosystem Research, and the Minnesota Environment and Natural Resources Trust Fund. PHACE support was provided by the USDA -Agricultural Research Service and CSREES (2008-35107-18655), the US Department of Energy's Office of Science (BER), and by the National Science Foundation (DEB# 1021559). YD is supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant XDB15010302) and the "100-Talent Program" of the Chinese Academy of Sciences.

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Supporting Information captions

A. Supplemental Methods and Materials

B. Supplemental Tables

Table S1 General information about six FACE experimental sites in this study

Table S2 Summary of samples used in this study

Table S3 Summary of plots and plot coordinates for all samples for this study.

Table S4 Effects of site, CO_2 and their interaction on soil properties

Table S5 The distance decay relationship at the functional gene and category levels

C. Supplemental Figures

Figure S1 Location of six FACE experimental sites in this study

Figure S2 The distance-decay rates within individual sites at aCO_2 and eCO_2

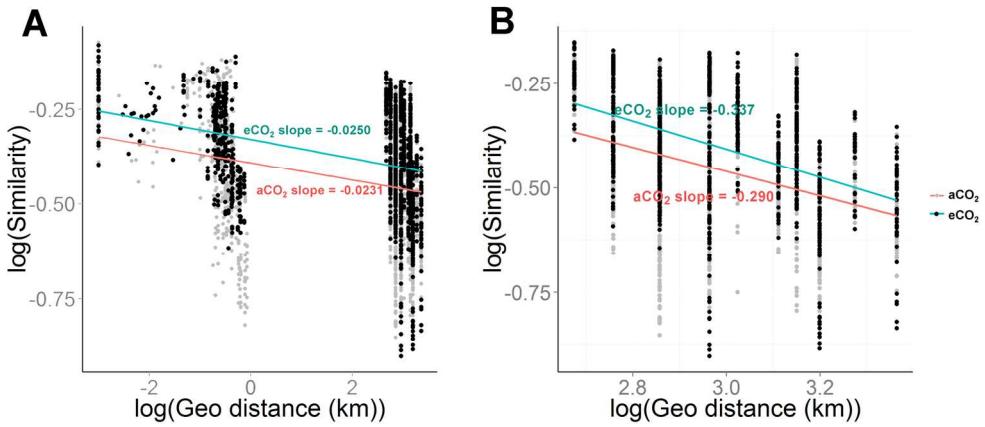
D. Supplemental references

Table 1 Relative importance of environmental factors contributing to the correlation by multiple regression on matrices (MRM) analysis.

	all (R^2 = 0.237, p = 0.001)		aCO_2 (R^2 = 0.228, p = 0.001)		eCO_2 (R^2 = 0.284, p = 0.001)	
	Coefficient	*p*	Coefficient	*p*	Coefficient	*p*
Log (Distance)	0.015	**0.001**	0.014	**0.001**	0.018	**0.001**
Log (Nitrate + 1)	0.025	**0.001**	0.029	**0.001**	0.002	0.499
Log (Ammonium)	0.117	**0.001**	0.119	**0.001**	0.138	**0.001**
Log (Total N)	-0.010	0.555	0.024	0.501	-0.057	0.084
Log (Total C)	0.046	**0.004**	0.026	0.355	0.086	**0.005**
Log (C:N ratio)	0.219	**0.001**	0.197	**0.001**	0.179	**0.001**

Figure captions

Figure 1 The distance-decay relationship of soil microbial communities from aCO_2 and eCO_2 samples. The x-axis is log (geographic distance) in kilometer and y-axis is log (similarity) calculated using the Sørensen method. Geographic distance was calculated from each two sites or plots based on plot coordinates (Table S3). (A) For all samples within and among six experimental sites, the slope of aCO_2 plots was -0.0231, and the slope of eCO_2 plots was -0.0250, and both slopes were significantly less than zero. The permutation test indicated these two slopes were significantly different ($t = 25.29$, $p < 0.001$, $df = 1998$). (B) For the geographic distances across six different sites, the slope of aCO_2 plots was -0.290, and the slope of eCO_2 plots was -0.337. The permutation test indicated these two slopes were significantly different as well ($t = 659.5$, $p < 0.001$, $df = 1998$).



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Supplementary Information

A. Supplemental Methods and Materials

B. Supplemental Tables

C. Supplemental Figures

D. Supplemental references

A. Supplemental Materials and Methods

Description, background and sampling of six sites/ecosystems

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.*, 2012b, He *et al.*, 2010b, Zhou *et al.*, 2010, Zhou *et al.*, 2011). In this study, we analyzed soil microbial communities sampled from 24 plots (12 for aCO_2 and 12 for eCO_2) with 16 species and without N addition in July 2007 when this site was exposed to eCO_2 for 10 years.

Duke. 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) 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). In this study, we analyzed soil microbial communities from 16 plots (8 each for aCO_2 and eCO_2) sampled in July 2008 when this site was exposed to eCO_2 for 15 years.

ORNL. The Oak Ridge National Laboratory (ORNL) FACE experiment is a sweetgum (Liquidambar styraciflua L.) plantation with four 25-m diameter plots with two eCO_2 (~ 544 ppm) and two aCO_2 (~ 376 ppm) (Norby *et al.*, 2001). The soil is classified as Aquic Haplidult with a pH of approximately 5.5-6.0, and a previous studies indicate no significant changes with the soil microbial community, soil pH, or soil moisture at eCO_2, while soil N availability declined significantly faster at eCO_2 (Austin *et al.*, 2009, Garten *et al.*, 2011), arising a question if the CO_2 fertilization effect is sustainable, especially in N limited forest ecosystems at eCO_2 conditions (Norby *et al.*, 2010). One possibility is that eCO_2-grwon trees may be able to access a larger inorganic N pool in deeper soil by increased root exploration at eCO_2 (Iversen *et al.*, 2011). In this study, 12 samples (6 each for aCO_2 and eCO_2) sampled in July 2008 were analyzed when this site was exposed to eCO_2 for 10 years.

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 & Wander, 2008), but eCO_2 did generally increase soil moisture for both SoyFACE and MaizeFACE experiments (Leakey *et al.*, 2009). 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). In this study, 24 soil samples were collected for CO_2 treatments (ambient and elevated CO_2) in October 2008 from SoyFACE plots and 24 soil samples in May 2009 from MaizeFACE plots at the depth of 0-15 cm. This site was exposed to eCO_2 for 7 or 8 years, respectively.

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 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 elevated CO_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 communities had an increased ability to decompose soil organic matter (SOM) compared with those from ambient CO_2 plots, suggesting positive feedbacks of soil microbial communities to this semi-arid ecosystem (Nie *et al.*, 2013). In this study, we only analyzed soil microbial communities from 10 plots (5 for aCO_2 and 5 for eCO_2) sampled in July 2008 when this site was exposed to eCO_2 for only 2 years.

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

DNA extraction, purification and quantitation

Soil DNA was extracted by freeze-grinding mechanical lysis as described previously(Zhou *et al.*, 1996), and was purified using a low melting agarose gel followed by phenol extraction. DNA quality was assessed by the ratios of 260 nm/280 nm, and 260/230 nm using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE), and final DNA concentrations were quantified with PicoGreen (Ahn *et al.*, 1996) using a FLUOstar Optima (BMG Labtech, Jena, Germany).

GeoChip analysis

GeoChip 3.0 was used to analyze all 110 soil DNA samples, and it contains about 28,000 probes covering approximately 57,000 gene variants from 292 functional gene families involved in C, N, P and S cycling, energy metabolism, antibiotic resistance, metal resistance and organic contaminant degradation (He *et al.*, 2010a), and GeoChip-based hybridization detection is considered quantitative (He *et al.*, 2012a). Details for target preparation, labeling, and GeoChip hybridization as well as data analysis are previously described (He *et al.*, 2010a, He *et al.*, 2012c, He *et al.*, 2010b). 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^{o}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 & Zhou, 2008), and raw data were pre-processed.

Statistical analysis

Multivariate and direct gradient analysis. Permutational multivariate analysis of variance (PERMANOVA)(Anderson, 2001) was used to evaluate the contribution of site/ecosystem and CO_2 as well as their interaction to microbial community variations with the Adonis function, and to partition sums of squares from a centroid based on a Bray-Curtis dissimilarity matrix implemented in R (R Development Core Team, 2012). It first calculates the distances among samples and then permutates the distance matrix for 999 times. Since our experiments were carried out in six experimental sites, randomization was only implemented within each site to control the effect across all sites. Significance tests were done using F-test based on sequential sums of squares from permutations. Different datasets of microbial communities generated with different analytical methods were used to examine whether different locations and/or elevated atmospheric CO_2 has significant effects on soil microbial communities. All three procedures (anosim, adonis and mrpp) were performed with the Vegan package in R.

Calculations of geographic distances and β-diversity

To create a geographic distance matrix between any two sampling sites, the geographic distance was calculated using latitudinal and longitudinal coordinates (Table S5) and the Haversine formula. β-diversity of soil microbial communities was analyzed using the Sørensen method. The distance-decay relationship was plotted as logarithmic similarity against logarithmic distance. A linear regression was used to obtain the slope. To examine the significance of distance-decay relationships, we tested if those slopes were significantly less than zero (Martiny *et al.*, 2011). Also, the significance of slopes between aCO_2 and eCO_2 was tested by permutation. It was considered as significant if p value < 0.05 in this study.

Multiple regression on matrices (MRM)

To identify the relative importance of multiple factors contributing to the distance-decay relationship, a multiple regression on matrices (MRM) was used (Legendre *et al.*, 1994). The partial regression coefficients of an MRM model give a measure of the rate of change in the soil microbial community similarity for variables of interests when other variables were held constant (Martiny *et al.*, 2011).

B. Supplemental Tables

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

Project	BioCON[a]	Duke[b]	ORNL[c]	MaizeFACE[d]	SoyFACE[d]	PHACE[e]
Site	Cedar Creek Ecosystem Science Reserve, MN	Duke Forest, NC	Oak Ridge, TN	Urbana-Champaign, IL		Cheyenne, WY
Ecosystem	Native C_3 grass, C_4 grass, legume, and forb species	Loblolly pine forest	Sweetgum plantation	Corn/Soybean rotation		Mixed grass prairie
Elevated CO_2	560 ppm	Ambient + 200 ppm	550 ppm	550 ppm		600 ppm
Other treatment	Plant diversity, and nitrogen	Soil nutrients	None	O_3, temperature, and drought		Temperature
Lat/Lon	45°24' N/ 93°12' W	35°58' N/ 79°5' W	35°54' N/ 84°20' W	40°2' N/88°13' W		41°11'N 104°54'W
Start-end year	1997-	1994-2010	1998 - 2009	2001-		2006-2013

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. ORNL FACE: http://face.ornl.gov/
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 Summary of samples used in this study (ambient CO_2 vs elevated CO_2). A total of 110 (55 for each CO_2 condition) were analyzed.

Site	BioCON	Duke	ORNL	MaizeFACE	SoyFACE	PHACE
Ecosystem	Grassland	Forest	Forest	Soybean	Corn	Grassland
Ring/block	6		4/5	8	8	2
Plot	24	16	12/15	24	24	10
Replicates	12	8	6	12	12	5
Depth	0-15 cm	0-10 cm	0-15	0-15 cm	0-15 cm	0-15 cm
Sub-total	24	16	12	24	24	10
Sampling time	July 2007	July 2008	July 2008	October 2008	May 2009	July 2008
eCO_2 exposure	10 years	15 years	10 years	7 years	7.5 years	2 years

Table S3 Summary of plots and plot coordinates for all samples for this study.

Site	CO_2	Plot	Latitude	Longitude
BioCON	Ambient	67	-93.19	45.40
BioCON	Ambient	69	-93.19	45.40
BioCON	Ambient	104	-93.19	45.40
BioCON	Ambient	107	-93.19	45.40
BioCON	Ambient	184	-93.19	45.40
BioCON	Ambient	188	-93.19	45.40
BioCON	Ambient	201	-93.19	45.40
BioCON	Ambient	222	-93.19	45.40
BioCON	Ambient	306	-93.18	45.40
BioCON	Ambient	344	-93.18	45.40
BioCON	Ambient	355	-93.18	45.40
BioCON	Ambient	358	-93.18	45.40
Duke	Ambient	1A	-79.09	35.98
Duke	Ambient	5A	-79.09	35.98
Duke	Ambient	6A	-79.09	35.98
Duke	Ambient	8A	-79.09	35.97
Duke	Ambient	9A	-79.09	35.98
Duke	Ambient	10A	-79.09	35.98
Duke	Ambient	11A	-79.09	35.98
Duke	Ambient	12A	-79.09	35.97
ORNL	Ambient	S4a	-84.34	35.90
ORNL	Ambient	S4b	-84.34	35.90
ORNL	Ambient	S4c	-84.34	35.90
ORNL	Ambient	S5a	-84.34	35.90
ORNL	Ambient	S5b	-84.34	35.90
ORNL	Ambient	S5c	-84.34	35.90
MaizeFACE	Ambient	1S1	-88.24	40.04
MaizeFACE	Ambient	1S2	-88.24	40.04
MaizeFACE	Ambient	1S3	-88.24	40.04
MaizeFACE	Ambient	4S1	-88.24	40.04
MaizeFACE	Ambient	4S2	-88.24	40.04
MaizeFACE	Ambient	4S3	-88.24	40.04
MaizeFACE	Ambient	10S1	-88.23	40.04
MaizeFACE	Ambient	10S2	-88.23	40.04
MaizeFACE	Ambient	10S3	-88.23	40.04
MaizeFACE	Ambient	11S1	-88.23	40.04
MaizeFACE	Ambient	11S2	-88.23	40.04
MaizeFACE	Ambient	11S3	-88.23	40.04
SoyFACE	Ambient	17S1	-88.23	40.04

SoyFACE	Ambient	17S2	-88.23	40.04
SoyFACE	Ambient	17S3	-88.23	40.04
SoyFACE	Ambient	24S1	-88.23	40.04
SoyFACE	Ambient	24S2	-88.23	40.04
SoyFACE	Ambient	24S3	-88.23	40.04
SoyFACE	Ambient	25S1	-88.23	40.04
SoyFACE	Ambient	25S2	-88.23	40.04
SoyFACE	Ambient	25S3	-88.23	40.04
SoyFACE	Ambient	32S1	-88.23	40.04
SoyFACE	Ambient	32S2	-88.23	40.04
SoyFACE	Ambient	32S3	-88.23	40.04
PHACE	Ambient	8	-104.89	41.20
PHACE	Ambient	12	-104.89	41.20
PHACE	Ambient	14	-104.89	41.20
PHACE	Ambient	22	-104.89	41.20
PHACE	Ambient	25	-104.89	41.20
BioCON	Elevated	23	-93.19	45.41
BioCON	Elevated	33	-93.19	45.41
BioCON	Elevated	45	-93.19	45.41
BioCON	Elevated	51	-93.19	45.41
BioCON	Elevated	147	-93.19	45.40
BioCON	Elevated	163	-93.19	45.40
BioCON	Elevated	173	-93.19	45.40
BioCON	Elevated	179	-93.19	45.40
BioCON	Elevated	250	-93.18	45.40
BioCON	Elevated	270	-93.18	45.40
BioCON	Elevated	272	-93.18	45.40
BioCON	Elevated	283	-93.18	45.40
Duke	Elevated	2A	-79.09	35.98
Duke	Elevated	3A	-79.09	35.98
Duke	Elevated	4A	-79.09	35.98
Duke	Elevated	7A	-79.09	35.97
Duke	Elevated	2B	-79.09	35.98
Duke	Elevated	3B	-79.09	35.98
Duke	Elevated	4B	-79.09	35.98
Duke	Elevated	7B	-79.09	35.97
ORNL	Elevated	S1a	-84.34	35.90
ORNL	Elevated	S1b	-84.34	35.90
ORNL	Elevated	S1c	-84.34	35.90
ORNL	Elevated	S2a	-84.34	35.90
ORNL	Elevated	S2b	-84.34	35.90
ORNL	Elevated	S2c	-84.34	35.90

MaizeFACE	Elevated	3S1	-88.24	40.04
MaizeFACE	Elevated	3S2	-88.24	40.04
MaizeFACE	Elevated	3S3	-88.24	40.04
MaizeFACE	Elevated	5S1	-88.23	40.04
MaizeFACE	Elevated	5S2	-88.23	40.04
MaizeFACE	Elevated	5S3	-88.23	40.04
MaizeFACE	Elevated	14S1	-88.23	40.04
MaizeFACE	Elevated	14S2	-88.23	40.04
MaizeFACE	Elevated	14S3	-88.23	40.04
MaizeFACE	Elevated	15S1	-88.23	40.04
MaizeFACE	Elevated	15S2	-88.23	40.04
MaizeFACE	Elevated	15S3	-88.23	40.04
SoyFACE	Elevated	20S1	-88.23	40.04
SoyFACE	Elevated	20S2	-88.23	40.04
SoyFACE	Elevated	20S3	-88.23	40.04
SoyFACE	Elevated	21S1	-88.23	40.04
SoyFACE	Elevated	21S2	-88.23	40.04
SoyFACE	Elevated	21S3	-88.23	40.04
SoyFACE	Elevated	28S1	-88.23	40.04
SoyFACE	Elevated	28S2	-88.23	40.04
SoyFACE	Elevated	28S3	-88.23	40.04
SoyFACE	Elevated	29S1	-88.23	40.04
SoyFACE	Elevated	29S2	-88.23	40.04
SoyFACE	Elevated	29S3	-88.23	40.04
PHACE	Elevated	3	-104.89	41.20
PHACE	Elevated	7	-104.89	41.20
PHACE	Elevated	11	-104.89	41.20
PHACE	Elevated	26	-104.89	41.20
PHACE	Elevated	27	-104.89	41.20

1

Table S4 The effect of site and CO_2 on soil properties analyzed by ANOVA.

	Site		CO_2		Site x CO_2	
	F value	P > F	F value	P > F	F value	P > F
Nitrate	52.253	**<0.0001**	2.9209	0.0906	6.435	**<0.0001**
Ammonium	14.0834	**<0.0001**	1.2869	0.2594	0.828	0.5328
Total nitrogen (TN)	16.3036	**<0.0001**	0.7447	0.3903	0.7665	0.5761
Total carbon (TC)	9.2797	**<0.0001**	0.8297	0.3646	0.3235	0.8978
C:N ratio	22.1813	**<0.0001**	1.0774	0.3018	0.3785	0.8624

Table S5 The significant distance decay relationships of key functional genes/categories at aCO_2 and eCO_2 and their significance of slopes (*p* values) between aCO_2 and eCO_2 by permutation tests.

Functional category and gene/enzyme	aCO_2		eCO_2		Significance (aCO_2 vs. eCO_2)
	r	Slope	r	Slope	
Carbon cycling	-0.292	-0.035	-0.321	-0.034	0.104
amyA	-0.190	-0.013	-0.293	-0.016	<0.001
Endochitinase	-0.168	-0.010	-0.268	-0.014	<0.001
Phenol oxidase	-0.159	-0.010	-0.212	-0.014	<0.001
Acc/Pcc	-0.154	-0.009	-0.232	-0.014	<0.001
Rubisco	-0.135	-0.010	-0.254	-0.014	<0.001
CODH	-0.112	-0.008	-0.269	-0.016	<0.001
Nitrogen cycling	-0.263	-0.027	-0.307	-0.027	0.036
nifH	-0.181	-0.010	-0.269	-0.013	<0.001
narG	-0.180	-0.010	-0.251	-0.012	<0.001
nirK	-0.185	-0.011	-0.272	-0.014	<0.001
nirS	-0.142	-0.009	-0.255	-0.013	<0.001
nosZ	-0.145	-0.008	-0.259	-0.014	<0.001
ureC	-0.076	-0.006	-0.243	-0.012	<0.001
Sulfur cycling	-0.298	-0.028	-0.338	-0.028	0.747
dsrA	-0.133	-0.008	-0.244	-0.012	<0.001
dsrB	-0.156	-0.011	-0.259	-0.015	<0.001
Phosphorus cycling	-0.233	-0.023	-0.288	-0.026	<0.001
ppx	-0.145	-0.008	-0.245	-0.012	<0.001
Energy process	-0.252	-0.020	-0.293	-0.019	<0.001
Cytochrome	-0.124	-0.008	-0.226	-0.012	<0.001
Phylogeny (*gyrB*)	-0.155	-0.009	-0.253	-0.013	<0.001
Organic Remediation	-0.202	-0.017	-0.307	-0.020	<0.001
alkK	-0.174	-0.014	-0.292	-0.019	<0.001
linB	-0.137	-0.011	-0.263	-0.017	<0.001
mdlA	-0.207	-0.015	-0.267	-0.015	0.457
nmoA	-0.223	-0.018	-0.273	-0.018	0.890
pcaG	-0.124	-0.008	-0.284	-0.016	<0.001
phn	-0.139	-0.008	-0.262	-0.011	<0.001
pimF	-0.187	-0.012	-0.286	-0.015	<0.001
tfdA	-0.234	-0.018	-0.303	-0.019	0.068
Metal Resistance	-0.247	-0.020	-0.320	-0.021	<0.001
arsC	-0.150	-0.010	-0.267	-0.016	<0.001
chrA	-0.132	-0.008	-0.268	-0.013	<0.001
copA	-0.190	-0.012	-0.259	-0.014	<0.001
czcA	-0.150	-0.011	-0.226	-0.012	<0.001
czcD	-0.182	-0.012	-0.271	-0.014	<0.001
terC	-0.161	-0.009	-0.272	-0.014	<0.001
zntA	-0.166	-0.011	-0.263	-0.014	<0.001

C. Supplemental Figures

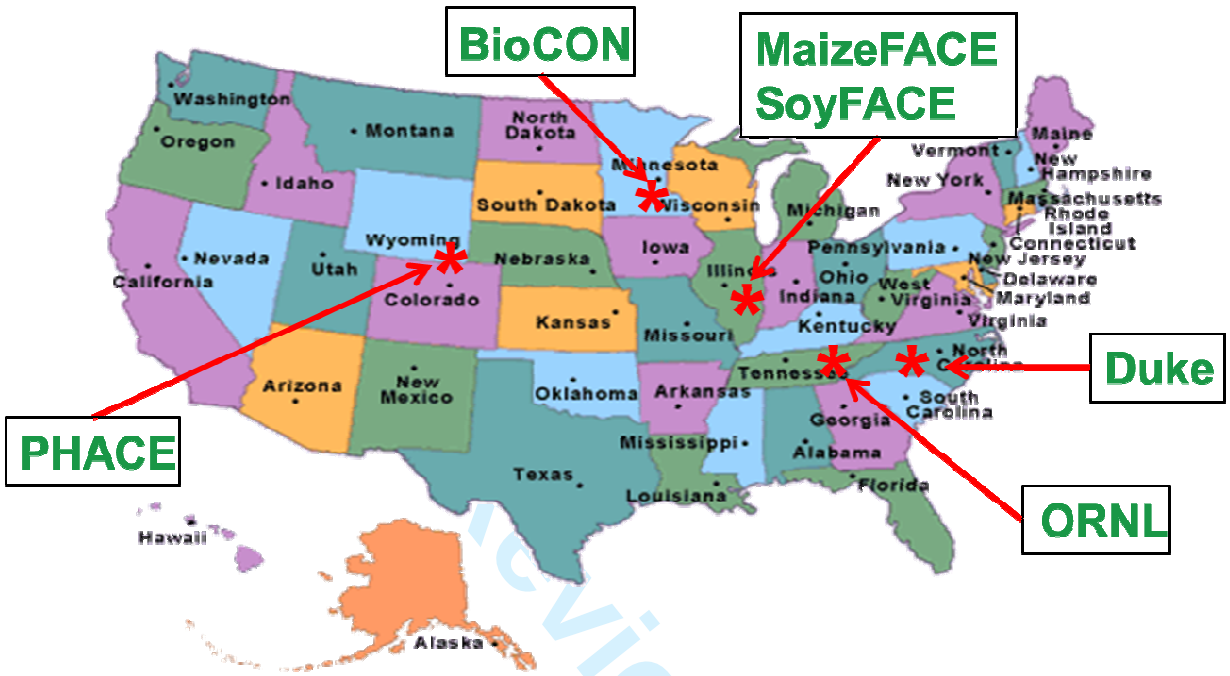

Figure S1 Location of six FACE experimental sites in this study. They are BioCON, Duke, ORNL, MaizeFACE, SoyFACE, and PHACE. Details about those sites are described in the Supplementary Information A (Supplemental Materials and Methods) and Table S1. Geographic distance ranges from less than 1.0 meter within a plot to a maximum of 2,302 km between the Duke Forest site and the PHACE site.

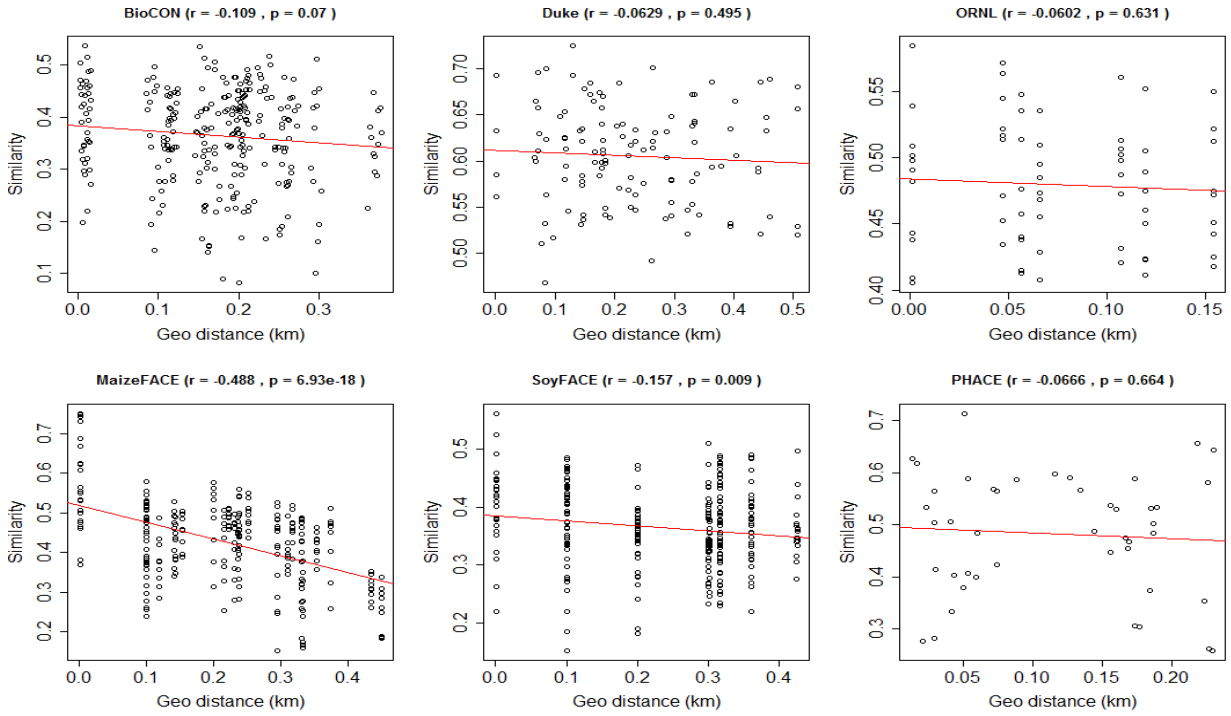

Figure S2 The distance decay rates of soil microbial communities within individual sites. Those rates were highly variable from significant distance relationships (MaizeFACE and SoyFACE), to no significant changes (BioCON, Duke, ORNL and PHACE). This may be largely due to the small distance scale, a limited number of plots for each site, and intertwining of multiple processes.

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