Global Change Biology

Global Change Biology (2016) **22**, 957–964, doi: 10.1111/gcb.13098

Elevated carbon dioxide accelerates the spatial turnover of soil microbial communities

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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 eCO_2 impacts on the geographic distribution of micro-organisms 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 with a slope of −0.0250 than at ambient CO_2 (aCO_2) with a slope of −0.0231 although it varied within each individual site, indicating that the spatial turnover rate of soil microbial communities was accelerated under eCO_2 at a larger 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 that may be especially important as global CO_2 continues to increase.

Keywords: elevated carbon dioxide, free air CO_2 enrichment, microbial community, spatial turnover rate, β-diversity

Received 7 May 2015 and accepted 27 August 2015

Introduction

Many lines of evidence have shown that elevated CO_2 (eCO_2) shifted the composition, structure and interaction of soil microbial communities and their ecosystem functions (Carney *et al.*, 2007; Blagodatskaya *et al.*, 2010; He *et al.*, 2010b, 2012a; Zhou *et al.*, 2011; Deng *et al.*, 2012; Van Groenigen *et al.*, 2014). Generally, eCO_2 increased plant growth and productivity, leading to an increased carbon (C) input into soil (Reich *et al.*, 2001; He *et al.*, 2010b, 2014), changed soil micro-environments, such as increased soil moisture (Adair *et al.*, 2009; He *et al.*, 2010b; Van Groenigen *et al.*, 2014; Xiong *et al.*, 2015), and stimulated microbial growth and activity (Blagodatskaya *et al.*, 2010; Kelley *et al.*, 2011; Van Groenigen *et al.*, 2014), but these responses have appeared to be highly variable in different ecosystems and sites (Carney *et al.*, 2007; Hungate *et al.*, 2009; Kelley *et al.*, 2011; Dunbar *et al.*, 2012), and no consensus has been reached. Moreover, 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 micro-organisms and their associated ecological processes across different ecosystems or habitats.

The distance-decay relationship (DDR) has been widely used to understand geographic patterns of biodiversity and community assembly mechanisms across a range of organisms at different spatial scales (Hanson *et al.*, 2012). It describes the declining pattern in community similarity with increases in geographic distance, which is usually measured by a linear regression between logarithmic β-similarities and logarithmic geographic distances (Nekola & White, 1999). The slope of

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linear regression reflects the species turnover rate, and it varies across different micro-organisms, regions and environmental gradients (Zhou *et al.*, 2008; Martiny *et al.*, 2011; Liang *et al.*, 2015). However, no experiments have been performed to examine such DDRs on a large (e.g., regional, global) scale at sites with parallel environmental treatments (e.g. aCO_2 vs. eCO_2). Thus, how experimental environmental manipulations (e.g. eCO_2) affect the turnover rate remains largely unclear.

Two ecological theories have been proposed to explain the distance-decay relationship in both macro- and micro-ecology. 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). 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 (Horner-Devine *et al.*, 2004; Fierer & Jackson, 2006; Zhou *et al.*, 2008, 2014; Martiny *et al.*, 2011; Finkel *et al.*, 2012; Hanson *et al.*, 2012). Also, 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). However, the detection of effects from such processes and their roles in maintaining biodiversity and supporting ecosystem functions are expected to be more sensitive with higher resolution markers (Horner-Devine *et al.*, 2004). For example, the 16S rRNA genes (that have a resolution largely at the genus/subfamily level) may not be ideal for detecting drift or mutation (Hanson *et al.*, 2012), while protein coding functional genes (resolution of species/strain level), such as *amoA* and *nifH*, may be better molecular markers for such purposes (Zhou *et al.*, 2008; Martiny *et al.*, 2011). Therefore, to understand the assembly mechanisms of soil microbial communities, it is necessary to comprehensively survey the distance-decay relationship with appropriate marker genes.

In this study, we hypothesized that the similarity of soil microbial communities would decline as distance increased largely due to environmental variation, and the turnover rate would be higher at eCO_2 than at aCO_2 as eCO_2 increased C inputs into soil (Reich *et al.*, 2001; He *et al.*, 2010b, 2014), changed soil micro-environments such as increased soil moisture (Adair *et al.*, 2009; He *et al.*, 2010b; Van Groenigen *et al.*, 2014; Xiong *et al.*, 2015), and stimulated microbial growth and activity (Blagodatskaya *et al.*, 2010; Kelley *et al.*, 2011; Van Groenigen *et al.*, 2014), thus increasing variation in microbial communities at eCO_2 (Webber *et al.*, 2011; Dunbar *et al.*, 2012; Hungate *et al.*, 2009; Carney *et al.*, 2007). To test those hypotheses, we analyzed the 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) representing a distance range between samples of <2.5 m to >2300 km using a comprehensive functional gene array, GeoChip 3.0 (He *et al.*, 2010a). Our results indicated that the spatial turnover rate of soil microbial communities and their functional genes was accelerated under eCO_2 across such a distance range.

Materials and methods

Experimental sites and sampling

A total of 110 soil samples were analyzed from six FACE experimental sites across the United States (Table S1). The BioCON (Biodiversity, CO_2, Nitrogen deposition) experiment site is a planted grassland site with CO_2, nitrogen fertilization and plant diversity treatments (Reich *et al.*, 2001). Here, we used the samples 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 had been exposed to eCO_2 for 10 years. The Duke Forest FACE experiment is a pine-dominated (>90% of basal area) forest ecosystem (Lichter *et al.*, 2008). 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 had been exposed to eCO_2 for 15 years. The Oak Ridge National Laboratory (ORNL) FACE experiment is a sweet gum (*Liquidambar styraciflua* L.) plantation with four 25-m-diameter plots (Norby *et al.*, 2010). We used 12 samples (six each for aCO_2 and eCO_2) sampled in July 2008 when this site had been exposed to eCO_2 for 10 years. The SoyFACE and MaizeFACE are typical corn-soybean rotation agro-ecosystems (Leakey *et al.*, 2009). Here, 24 soil samples were collected for CO_2 treatments (12 ambient and 12 elevated CO_2) in October 2008 from SoyFACE plots and 24 soil samples in May 2009 from MaizeFACE plots. These sites had been exposed to eCO_2 for 7 or 8 years, respectively. The PHACE (Prairie Heating and Carbon Dioxide Enrichment) experiment includes two levels of CO_2 (ambient 400 ppm vs. elevated 600 ppm) and two temperature (ambient vs. elevated with 1.5–3.0 °C warmer day/night) regimes in 20 (3.3 m diameter) circular plots (Dijkstra *et al.*, 2010). We only analyzed soil microbial communities from 10 plots (5 each for aCO_2 and eCO_2) sampled in July 2008 when this site had been exposed to eCO_2 for 2 years. As the distribution of the sampling plots and their sizes were different across six sites, the distance among replicate samples within each site varied from 2.5 to 864 meters. Details about ecosystems, sampling plot numbers and sampling time are listed in Table S2.

Soil properties measurement, DNA extraction and GeoChip hybridization

Soil NO_3-N and NH_4-N were extracted with 1 M KCl solution and quantified by a Flow Injection Autoanalyzer (LACHAT,

1994), and total soil C and nitrogen (N) were determined using a LECO Truspec dry combustion carbon analyzer (Nelson & Sommers, 1996). Soil DNA was extracted by freeze-grinding mechanical lysis from each sample as described previously (Zhou *et al*., 1996), and the final DNA concentrations were quantified with PicoGreen (Ahn *et al*., 1996) using a FLUOstar Optima (BMG Labtech, Jena, Germany).

The functional gene micro-array, GeoChip 3.0, was used to analyze key functional genes involved in important ecological processes, and details of target preparation, labeling, and micro-array hybridization as well as data analysis were described previously (He *et al*., 2010b, 2012b). The GeoChip 3.0 contained about 28,000 probes from 292 functional gene families involved in carbon, nitrogen, phosphate (P), and sulfur (S) cycling, energy metabolism, metal resistance, and organic contaminant degradation (He *et al*., 2010a). Most functional gene families had specific probes derived from 100 to 2000 species/genera; thus, the GeoChip can detect multiple subsets of micro-organisms with certain ecological functions. Also, the phylogenetic marker gene for DNA gyrase (*gyrB)* gene was integrated into this GeoChip and can also be used to detect specific micro-organisms.

Preprocessing of GeoChip data

After micro-array slides were scanned by a NimbleGen MS200 scanner (Madison, WI, USA), the signal intensities were digitized from the raw images data, which were uploaded to the laboratory micro-array processing pipeline (http://ieg.ou.edu/microarray/) and preprocessed as described previously (Deng & He, 2014; Li *et al*., 2014). Briefly, spots with a signal-to-noise ratio (SNR) > 2.0, and signal intensities greater than 1000 were considered as positive signals (He & Zhou, 2008). The raw signal was then log-transformed and normalized through multiple steps using both internal and external standards (Deng & He, 2014; Li *et al*., 2014). The final normalized signals were listed with each row as a probe representing a functional gene in certain species and each column as an experimental sample for statistical analysis.

Statistical analysis

Regular analysis of variance (ANOVA) was implemented to measure the differences of soil properties among six experimental sites. All measured soil properties including nitrate, ammonium, total C, total N and C:N ratio were compared in crossover (CO_2 × site) tests. 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 permutated the distance matrix for 999 times. As our experiments were carried with variable numbers of samples originating from within six experimental sites, randomization was only implemented within each site to control the effect across all six sites. Significance tests were done using the *F*-test based on sequential sums of squares from permutations. All three procedures (*anosim*, *adonis* and *mrpp*) were performed with the Vegan package in R (Dixon, 2003).

To create a geographic distance matrix between any two sampling sites, the geographic distance was calculated using latitudinal and longitudinal coordinates of each sampling position (Table S3) through the R package Imap (Wallace, 2012). The β-diversity of soil microbial communities was analyzed using the Sørensen method. The distance-decay relationship was plotted as logarithmic similarity against logarithmic distance and a linear regression performed to obtain the slope. To examine the significance of distance-decay relationship curves, we tested whether those slopes were significantly less than zero with 1000 permutations (Martiny *et al*., 2011). Also, the significance of slopes between aCO_2 and eCO_2 was tested by permutation. It was considered significant only if $P < 0.05$ in this study.

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 gave a measure of rate of change in microbial community similarity for variables of interest when other variables were held constant (Martiny *et al*., 2011). The R package *ecodist* was used in MRM calculations (Goslee & Urban, 2007).

Results and Discussion

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 treatment ($P > 0.05$), and that their interaction was only significant ($P < 0.001$) for soil nitrate (Table S4). Under eCO_2, previous studies have shown that soil moisture increased in five of six sites except ORNL, and soil pH increased in BioCON but remained unchanged in other five sites (Adair *et al*., 2009; Leakey *et al*., 2009; Dijkstra *et al*., 2010; Norby *et al*., 2010).

Based on GeoChip hybridization signals from all 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 (Fig. S1), 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 (Fig. 1a) at the overall scale across all sites (pairwise against each other). Permutation tests also indicated that these two slopes were significantly ($P < 0.001$) different. When the distance-decay rates were calculated between any two samples across six sites (without consideration of rates within each site), much steeper slopes were observed for both eCO_2

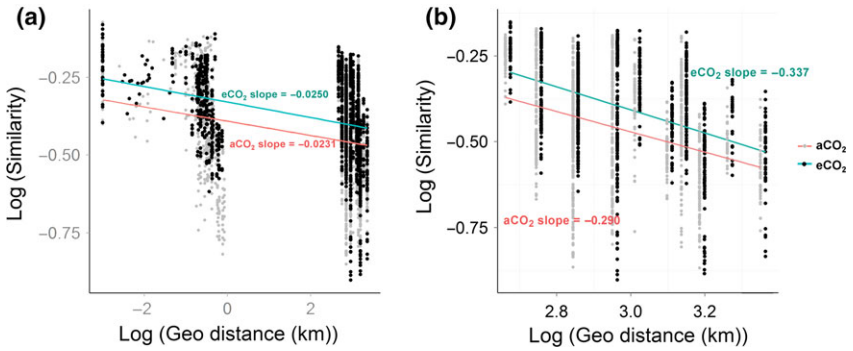

Fig. 1 The distance-decay relationship of soil microbial communities from aCO_2 and eCO_2 samples. The *x*-axis is log (geographic distance) in kilometer and *y*-axis is log (similarity) calculated using the Sørensen method. Geographic distance was calculated from each two sites based on plots coordinates (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, indicating significant patterns of distance-decay relationship. The permutation test indicated these two slopes were significantly different ($t = 25.29$, $P < 0.001$, df = 1998). (b) For the geographic distances of any two samples among 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).

(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 (Fig. 1b). These combined results suggested a higher distance-decay rate of soil microbial communities under eCO_2 compared with aCO_2 across six experimental sites.

Partial multiple regression on matrices (MRM) (Martiny *et al*., 2011) further identified the relative importance of distance and soil properties contributing to such distance-decay relationships. For the overall MRM model with all selected variables (distance, total C, total N, nitrate, ammonium and C:N ratio), 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 among the measured soil properties, C:N ratio and ammonium were major drivers of the *β*-diversity of soil microbial communities under both eCO_2 and aCO_2 conditions.

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 relationships for specific functional populations. The results showed significant distance-decay relationships at the functional category and the functional gene family levels under aCO_2 or eCO_2 (Table 2). 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 the phylogenetic marker, *gyrB*, also showed

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**

Bold indicates significant value $P < 0.01$.

Table 2 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

	aCO_2		eCO_2		
Functional category and gene/enzyme	*r*	Slope	*r*	Slope	Significance (aCO_2 vs. eCO_2)
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

the same trend (Table 2). The results suggest that eCO_2 thus also accelerated the spatial turnover rate of functional subcommunities at the functional category and functional gene family level.

Understanding the mechanisms that generate and maintain biodiversity is the key to predict 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 especially so at the larger scales. The soil C:N ratio and ammonium could largely contribute to the microbial DDR under both aCO_2 and eCO_2 conditions. Our results provide new hypotheses for further understanding their assembly mechanisms.

It was hypothesized that the similarity of soil microbial communities would decline as geographic distance increased, and the changes of distance-decay relationship would be largely driven by environmental variation. Consistent with this hypothesis, our results were 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, with the largest contribution from soil C:N ratio (Table 1). Indeed, previous studies revealed the soil C:N ratios had close links with bacterial and fungal growth as well as total biomass (Cleveland & Liptzin, 2007; Rousk & Bååth, 2007; Fierer et al., 2009); however, the mechanisms by which C:N ratios impact the microbial community assembly need further studies.

Why was a higher distance-decay rate observed at eCO_2? Many studies have demonstrated that eCO_2 caused inconsistent microbial responses in different ecosystems and the mechanisms behind them might be divergent (Carney et al., 2007; Hungate et al., 2009; He et al., 2010b; Kelley et al., 2011; Deng et al., 2012; Dunbar et al., 2012). Here, we propose a few possible mechanisms that could contribute to a higher turnover rate at eCO_2. First, soil C inputs increase at eCO_2 (Van Groenigen et al., 2014), and this may drive micro-organisms from oligotroph-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. It is also possible that additional C resources in soil may introduce more stochastic effects/processes in the microbial community, leading to higher β-diversity by randomness among different sites as our previous study showed in a groundwater system (Zhou et al., 2014). In addition, with increased substrates in soil, the generation time may be shorter (e.g., more generations in a given time). As generation time is a critical factor in understanding evolutionary rates and mechanisms, it can result in increased numbers of mutations for selection to act on. 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 (unpublished data), 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 showed that nitrate was significantly different among sites and under $CO_2 \times$ site (Table S4), and nitrate was a significant contributor to the distance-decay relationship under aCO_2 but not under eCO_2. Additionally, C:N ratio and ammonium were found to be the most important contributors to this relationship under both CO_2 conditions, indicating eCO_2 did influence soil nitrate availability and distance-decay relationships at eCO_2. Third, soil moisture generally increases at eCO_2 (He et al., 2010b; Van Groenigen et al., 2014), which may stimulate microbial growth and activity, especially micro-organisms involved in C decomposition and N cycling (He et al., 2010b; Van Groenigen et al., 2014), increase anaerobic functional processes (e.g., denitrification, methanogenesis), and enhance microbial access to growth substrates and nutrients (Blagodatskaya et al., 2010; He et al., 2010b, 2014; Kelley et al., 2011; Van Groenigen et al., 2014), further driving the divergence of soil microbial communities under eCO_2. All of these possible mechanisms could shape soil microbial communities in different ecosystems generating the distance-decay relationship and increase its spatial turnover rate under eCO_2 across those six sites while simultaneously driving communities to divergent responses. Dispersal and mutation may also increase at eCO_2, but they may not significantly impact the distance-decay relationship across those six sites. Due to these different mechanisms, the changes of microbial communities may thus be of differing magnitude or even in different directions among disparate ecosystems and the decline of β-diversity across a large distance scales accelerated.

The distance-decay relationship also varied within each individual site from significant DDRs for two sites (MaizeFACE and SoyFACE) to insignificant DDRs for the other sites (Fig. S1). This contrasts a previous study of ammonium-oxidizing bacterial communities within salt marsh sediments, which showed a significant distance-decay relationships within and across sites (Martiny et al., 2011). Possible reasons may include a narrow range distance scale examined, limited number of experimental plots for each site, and/or other unaccounted factors and processes. For example, at local scales, dispersal may entirely counteract compositional differentiation imposed by drift and selection, eliminating or reducing the magnitude and significance of the distance-decay relationship (Hanson et al., 2012).

Many microbial biogeography studies were performed in environmentally controlled ecosystems, which are expected to better reveal the distance-decay relationship and community assembly mechanisms (Bell, 2010). Some natural microbial biogeography studies were conducted in similar habitats/ecosystems, such as salt marsh sediments (Horner-Devine et al., 2004; Martiny et al., 2011), the fungi within *Pinaceae*-dominated forest soils (Talbot et al., 2014), rainforest-conversional agricultural soils (Rodrigues et al., 2013), and phyllosphere on *Tamrix* Trees (Finkel et al., 2012), while other studies were implemented across known

gradients of environmental change, such as salinity gradient (Logares *et al*., 2013) and eutrophication gradients in peatlands (Vilar *et al*., 2014). All these studies provide significant insights into our understanding of microbial biogeography and their regulatory mechanisms. However, in most cases, highly similar ecosystems are extremely difficult to find, and almost no identical ecosystems exist in the natural environment. As a result, most studies of the distance-decay relationship of microbial communities have been accomplished 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 as well.

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 divergent microbial responses and changed micro-environments among different ecosystems, providing new framework for further understanding their assembly mechanisms. Our results imply that eCO_2 may affect geographic patterns of soil microbial communities in the future eCO_2 environment.

Acknowledgements

This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant XDB15010302) and '100 Talents' program of Chinese Academy of Sciences to Ye Deng. The experiments were conducted 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, DEB-0620652, 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). The authors declared no conflict of interest in this paper.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

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.
Figure S1. The distance-decay rates within individual sites at aCO_2 and eCO_2.