environmental microbiology

Environmental Microbiology (2015) **17**(3), 566–576 doi:10.1111/1462-2920.12398

The interactive effects of soil transplant into colder regions and cropping on soil microbiology and biogeochemistry

Shanshan Liu,[1] Feng Wang,[2,3] Kai Xue,[4] Bo Sun,[2] Yuguang Zhang,[5] Zhili He,[4] Joy D. Van Nostrand,[4] Jizhong Zhou[1,4,6] and Yunfeng Yang[1]*

[1]*State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing, China.*

[2]*State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China.*

[3]*University of Chinese Academy of Sciences, Beijing, China.*

[4]*Institute for Environmental Genomics, Department Microbiology and Plant Science, University of Oklahoma, Norman, OK, USA.*

[5]*Institute of Forestry Ecology, Environment and Protection, Key Laboratory of Forest Ecology and Environment of State Forestry Administration, Chinese Academy of Forestry, Beijing, China.*

[6]*Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA.*

Summary

Soil transplant into warmer regions has been shown to alter soil microbiology. In contrast, little is known about the effects of soil transplant into colder regions, albeit that climate cooling has solicited attention in recent years. To address this question, we transplanted bare fallow soil over large transects from southern China (subtropical climate zone) to central (warm temperate climate zone) and northern China (cold temperate climate zone). After an adaptation period of 4 years, soil nitrogen components, microbial biomass and community structures were altered. However, the effects of soil transplant on microbial communities were dampened by maize cropping, unveiling a negative interaction between cropping and transplant. Further statistical analyses with Canonical correspondence analysis and Mantel tests unveiled annual average temperature, relative humidity, aboveground biomass, soil pH and NH_4^+-N content as environmental attributes closely correlated with microbial functional structures. In addition, average abundances of *amoA-AOA* (ammonia-oxidizing archaea) and *amoA-AOB* (ammonia-oxidizing bacteria) genes were significantly ($P < 0.05$) correlated with soil nitrification capacity, hence both AOA and AOB contributed to the soil functional process of nitrification. These results suggested that the soil nitrogen cycle was intimately linked with microbial community structure, and both were subjected to disturbance by soil transplant to colder regions and plant cropping.

Received 17 June, 2013; revised 8 January, 2014; accepted 8 January, 2014. *For correspondence. E-mail yangyf@tsinghua.edu.cn; Tel. (+86) 010 62784692; Fax (+86) 010 62785687.

Introduction

Global change induces shifts in microbial community composition, which in turn alters microbially mediated greenhouse gas emissions in nature (Hansen *et al.*, 2006; Walther, 2010). By using a strategy of soil transplant into warmer regions to simulate climate warming, it has been shown that soil microbial community structure and community functions were altered (Vanhala *et al.*, 2011), which was consistent with a number of studies showing that climate warming alters microbial communities (Petchey *et al.*, 1999; Rinnan *et al.*, 2007; Zhou *et al.*, 2012). It is also of interest to investigate the effects of soil transplant into colder regions, which may contribute to our understanding of climate cooling, since the impact of climate cooling on an ecosystem can be at a scale comparable to that of climate warming (McAnena *et al.*, 2013). Although long-term cooling worldwide may have a low probability of occurrence, it could occur locally or temporally, and may have large and devastating consequences (Alley *et al.*, 2003). For instance, the cooling of sea surface temperatures in the centre of the North Pacific Ocean in 1977 has resulted in serious consequences for marine ecosystems (Hare and Mantua, 2000).

In particular, we are interested in understanding whether and how the composition and functional potentials of the microbial community are linked to community metabolism. Although a growing body of studies has demonstrated that microbial community composition plays an essential role in biogeochemical cycles (Falkowski *et al.*, 2008), the nature and quantification of this linkage is

neither consistent nor tractable. This knowledge gap has resulted in the disputable assumption of global climatic models that microbial community composition is irrelevant to global climate change (Reed and Martiny, 2007).

In this study, we specifically addressed the following questions: (i) whether and how soil attributes, microbial biomass and community structures responded to soil transplant into colder regions, (ii) whether and how agricultural cropping modified the effect of soil transplant and (iii) whether changes in microbial communities were linked to changes in environmental attributes related to the disturbances of soil transplant and cropping. To this end, we transplanted soil in 2005 between three long-standing agricultural stations – from Yingtan, Jiangxi Province, southern China (site S) to Fengqiu, Henan Province of central China (site SC) and to Hailun, Heilongjiang Province of northern China (site SN), exposing soil from the subtropical climate zone to warm and cold temperate climate zones respectively. The soil type studied was ferric acrisol in the FAO (the Food and Agriculture Organization of the United Nations) classification system (red soil), which is widely distributed across Asia, Africa, North America and South America.

Table 1. Environmental attributes of S, SC and SN samples.

	S[a]	SC	SN
Climate attributes			
Annual average T[b] (°C)	**18.04[a]**	**14.05**	**1.46[b]**
Rainfall (mm)	**132.41[a]**	**128.8**	**60.4[b]**
Relative humidity (%)	**77.80[b]**	**80.05**	**85.13[a]**
Soil physical-chemical attributes			
Soil T (°C)	29.25[a]	30.07[a]	27.37
pH	5.97[a]	6.54[a]	5.80[a]
Moisture (%)	**21.62[a]**	**9.59**	**7.45[b]**
Organic matter (g kg^{-1})	9.92[a]	10.05[a]	8.91[a]
TN (g kg^{-1})	0.64[a]	0.69[a]	0.59[a]
TP (g kg^{-1})	0.44[a]	0.49[a]	0.34
TK (g kg^{-1})	9.86[a]	13.04[a]	9.95[a]
AN (mg kg^{-1})	**52.81[a]**	**49.51[a]**	**39.61**
AP (mg kg^{-1})	21.06[a]	17.14[a]	12.94[a]
AK (mg kg^{-1})	195[a]	227.5[a]	186.67[a]
NH_4^+-N (mg kg^{-1})	**2.98[a]**	**2.46**	**2.47**
NO_3^--N (mg kg^{-1})	**12.06**	**35.07[a]**	**7.75[b]**
Soil ecoprocesses			
Nitrification capacity (%)	9.05[a]	9.09[a]	9.78[a]
Soil CO_2 efflux (umolCO_2/(m^2 $s)^{-1}$)	1.02[a]	1.79[a]	1.38[a]

NH_4^+-N, ammonium; NO_3^--N, nitrate; T, temperature.
a. S: soil samples in southern China (Yintan); SC: soil samples transplanted from southern China to central China (Fengqiu); SN: soil samples transplanted from southern China to northern China (Hailun).
b. Attributes with significant differences are shown in bold font.

Results

Effects of soil transplant on soil attributes

Although climate regimes differed substantially between the transplant sites and the control site, most of the soil biogeochemical attributes, including organic matter, total nitrogen (TN), total phosphorus (TP) and total potassium (TK), remained unaltered 4 years after soil transplant (Table 1). However, NH_4^+-N content decreased from 2.98 to 2.46–2.47 mg kg^{-1} at sites SC and SN, alkali-hydrolysable nitrogen (AN) decreased from 52.81 mg kg^{-1} to 49.51 mg kg^{-1} at site SC and 39.61 mg kg^{-1} at site SN but NO_3^--N content increased from 12.06 to 35.07 mg kg^{-1} at site SC and decreased to 7.75 mg kg^{-1} at site SN. These results indicated that soil nitrogen components were more sensitive than other elemental nutrients in response to soil transplant. The effects of nitrogen components were further explored by correlation analyses. The results showed that annual average temperature and rainfall were positively correlated with TN ($r = 0.47$–0.48, $P < 0.05$) and AN ($r = 0.67$–0.73, $P < 0.002$), and soil moisture was correlated with AN ($r = 0.52$, $P = 0.03$). Thus, these climatic attributes imposed an effect on soil nitrogen, which was consistent with previous reports (Shaw and Harte, 2001; Rowe *et al.*, 2012).

Effects of soil transplant on soil microbial communities

The functional composition of soil microbial communities was analysed using a high-throughput metagenomics tool named GeoChip. Microbial functional diversity, determined by Simpson's index, decreased from 2094.5 at site S to 1811.0 at site SC and to 1685.0 at site SN (Supporting information Fig. S1). In addition, site S, SC and SN samples were well separated by detrended correspondence analysis (DCA) (Fig. 1), which was confirmed by the dissimilarity test of Adonis, showing that the differences were significant ($P < 0.001$) (Table 2).

The results of phospholipid fatty acid (PLFA) analysis showed that soil at site SC had a lower total microbial biomass (11.79 nmol g^{-1} dry soil) and fungal biomass (0.71 nmol g^{-1} dry soil) than soil at site S (21.28 nmol total microbial biomass/g dry soil and 1.77 nmol fungal biomass/g dry soil), while bacterial biomass remained unchanged between these two sites. In contrast, the total microbial, fungal and bacterial biomass remained unchanged at site SN (Supporting information Fig. S2). The ratios of bacteria to fungi were increased at both site SC (2.2 fold, $P = 0.004$) and site SN (1.6 fold, $P = 0.06$) (Fig. 2A). These results revealed the differential sensitivity of bacteria and fungi to cooler and drier climate regimes.

To examine whether environmental attributes influenced bacterial and fungal biomass, we analysed Pearson correlations between bacterial/fungal ratios and environmental attributes. As shown in Fig. 2B, the ratios of bacteria to fungi were negatively and positively correlated with soil moisture ($r = -0.61$, $P = 0.01$) and pH ($r = 0.52$, $P = 0.02$) respectively. These two attributes

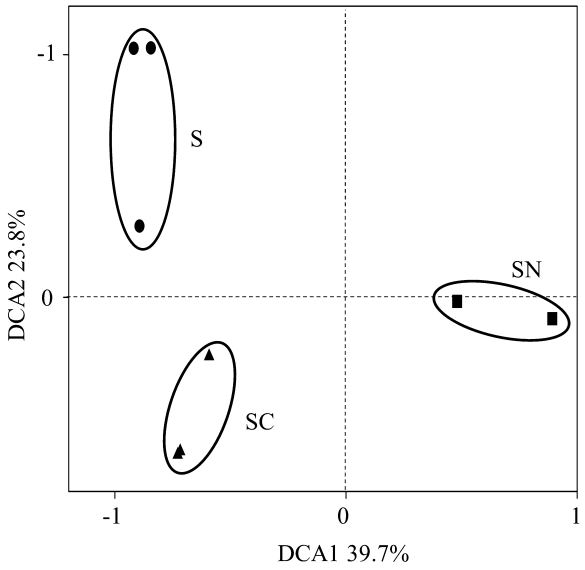

Fig. 1. The effect of soil transplant into colder regions on microbial community functional structure revealed by detrended correspondence analysis in bare fallow samples. S: soil samples in southern China (Yintan); SC: soil samples transplanted from southern China to central China (Fengqiu); SN: soil samples transplanted from southern China to northern China (Hailun).

were both linked to the relative abundance of bacterial and fungal communities; however, their effects were felt in opposite directions.

Effects of soil transplant on selected functional genes

Next, we focused on functional genes present only at both transplant sites, which might be important for microbial adaptation to new environments. This gene group included 89 genes, many of which were derived from *Proteobacteria* and *Actinobacteria* prevalent in soil (Roesch *et al*., 2007). In contrast, no genes were derived from fungi, and only three genes were derived from archaea. Most of these genes are involved in organic remediation related to the degradation of complex carbon substrates (34.83%), carbon cycling (21.35%), nitrogen cycling (7.87%), antibiotic resistance (8.99%) and metal resistance (16.85%) (Supporting information Table S1). Compared with the overall percentages of gene categories in the control soil, genes related to organic remediation and the carbon cycle were overrepresented (increased by 7.38% and 1.86% respectively), implying that carbon cycling mediated by microbes was required at the transplant sites. In contrast, nitrogen cycling and metal resistance genes were underrepresented at the transplant sites (decreased by 3.82% and 5.98% respectively).

A total of 582 genes were exclusively detected at site S (Supporting information Table S2). Among these genes,

Table 2. The statistical test of Adonis[a] to analyse differences in soil microbial community compositions measured by GeoChip.

	GeoChip	
Method	Statistic R^2	*P*-value
S vs SC	0.786	0.001
S vs SN	0.715	0.001
SC vs SN	0.703	0.001

a. Adonis: non-parametric multivariate analysis of variance with the Adonis function.

84 were carbon degradation genes. Interestingly, 15 of these carbon degradation genes were derived from fungi such as *Eurotiomycetes* and *Agaricomycetes*, suggesting that the carbon degradation potential of these fungal species was reduced. There might be a shift in the soil carbon use profile from recalcitrant carbon to labile carbon because considerably more genes involved in degrading recalcitrant carbon (cellulose, chitin and lignin)

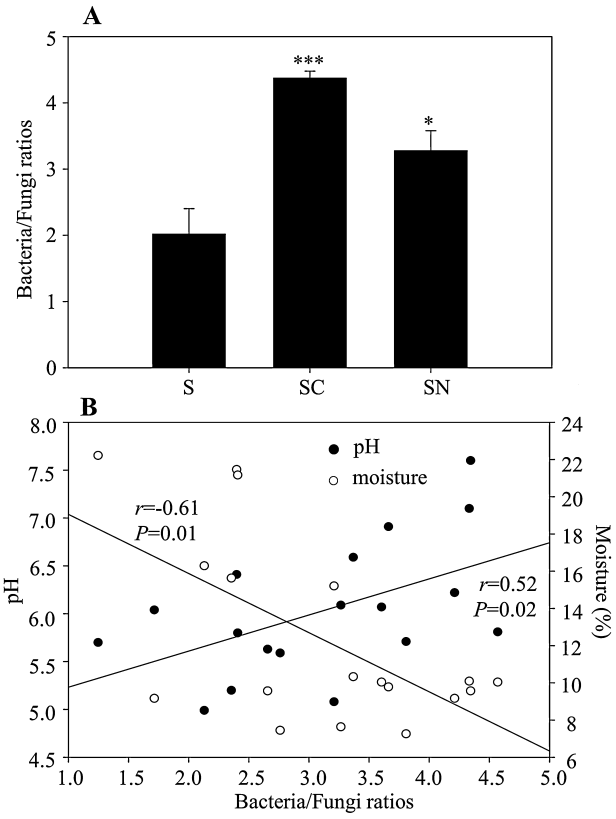

Fig. 2. A. Changes in bacterial/fungal ratios in bare fallow samples. Significant differences between S and SC or between S and SN were analysed by two-tailed *t*-tests with two-sample equal variance and indicated by '*' for $P < 0.1$ and '***' for $P < 0.01$ respectively. Symbols as in Fig. 1. B. Pearson correlations between ratios of bacteria to fungi and pH and soil moisture. The ratios were positively correlated with pH ($P = 0.02$) and negatively correlated with soil moisture ($P = 0.01$). Filled and open circles represent soil pH and moisture respectively.

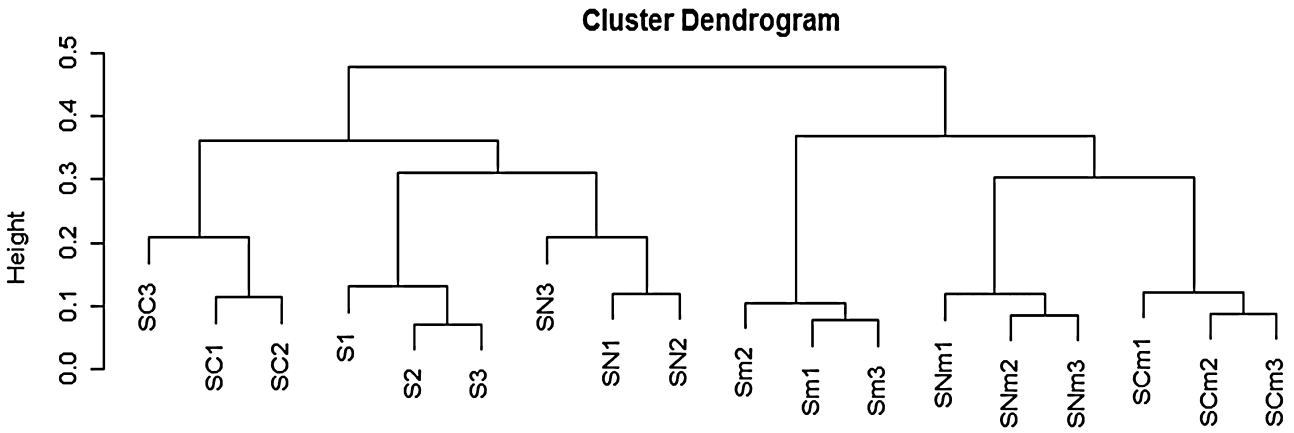

Fig. 3. Hierarchical clustering analysis of microbial communities for all soil samples in triplicate based on GeoChip data. Results were generated in R 2.12.2 with Vegan package. Symbols as in Fig. 1; m: maize cropping.

disappeared after soil transplant when compared with those involved in degrading labile carbon (starch and hemicellulose). For carbon fixation, 14.9% of detected *CODH* genes disappeared after soil transplant, which was higher than the disappearance of genes *pcc* (6.3%) and *rubisco* (12.3%).

The interactive effect of soil transplant and maize cropping

GeoChip data from samples under the dual factors of soil transplant and maize cropping (SCm and SNm) was significantly ($P < 0.04$) different from that of a single factor (Supporting information Table S3), suggesting that maize cropping interacted with the effect of soil transplant on microbial communities. This was verified by DCA (Supporting information Fig. S3) and hierarchical clustering analysis (Fig. 3), which showed that samples were grouped by their respective treatment(s).

To explore the possibility of interactions between climate cooling and maize cropping, we calculated the additive effect of soil transplant and maize cropping on microbial alpha-diversities with the assumption that the interactive effect was zero. The observed effect was considerably lower than the additive effect (Supporting information Fig. S4), indicating a negative interaction between soil transplant and maize cropping.

Relationships between microbial functional genes and environmental attributes

Canonical correspondence analysis was performed to reveal the relationships between microbial community composition and environmental attributes. In this analysis, the environmental attributes with and without maize cropping were all included (Table 1 and Supporting information Table S4). Six environmental attributes (annual average temperature, pH, relative humidity, AN, NH_4^+-N and aboveground biomass) were selected based on their variance inflation factors (VIFs) (See Methods and Materials for details). The results indicated that climate conditions (annual average temperature and relative humidity), soil conditions (pH, NH_4^+-N and AN) and vegetation (aboveground biomass) were major factors influencing microbial community structure (Fig. 4). These results were further verified by Mantel tests (Table 3).

We performed correlation analyses to examine the influence of environmental attributes on individual functional genes. A total of 134 genes were significantly ($P < 0.01$) correlated with annual average temperature

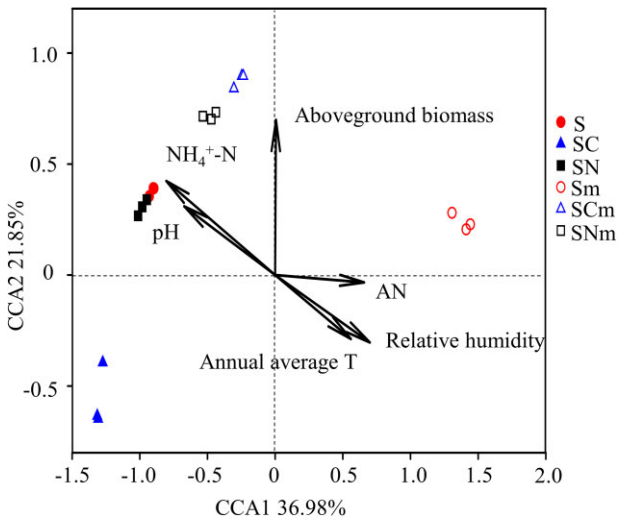

Fig. 4. CCA of the GeoChip data (symbols) and environmental attributes (arrows). The percentage of variation explained by each axis is shown. The CCA model is significant ($P = 0.005$). Symbols as in Fig. 3; Annual average T: annual average temperature; AN: alkali-hydrolysable nitrogen; NH_4^+-N: ammonium.

Table 3. Correlations between all detected genes and environmental attributes by Mantel test.

	Statistic r	*P*-value
Annual average T	0.156	0.062
Rainfall	0.046	0.301
Relative humidity	0.144	0.048*[a]
Soil T	0.0097	0.389
pH	0.358	0.012*
Moisture	0.079	0.19
Organic matter	0.033	0.334
TN	0.131	0.119
TP	–0.074	0.622
TK	0.068	0.182
AN	0.216	0.036*
AP	–0.050	0.611
AK	–0.087	0.677
NH_4^+-N	0.734	0.001**
NO_3^--N	0.148	0.1
Grain weight	0.092	0.219
Aboveground biomass	0.230	0.067

T, temperature; NH_4^+-N, ammonium; NO_3^--N, nitrate.
a. Asterisk indicates significant differences. '*', $P < 0.05$; '**', $P < 0.01$.

(Supporting information Table S5). The correlations were predominantly positive, suggesting that lower temperature decreased gene abundance and hence microbial functional potentials at our study sites. There were several ribulose-1, 5-bisphosphate carboxylase/oxygenase (*rubisco*) genes for the Calvin cycle and carbon monoxide dehydrogenase (*CODH*) genes for the reductive acetyl-CoA pathway correlated with temperature, suggesting that these carbon fixation pathways were sensitive to decreased temperature. In contrast, no propionyl-CoA carboxylase (*pcc*) gene for the 3-hydroxypropionate cycle was correlated with temperature, despite the presence of a large number of *pcc* probes on GeoChip. A number of genes involved in the methane cycle and labile carbon degradation were also identified, for instance, *pmoA* for methane oxidation, which has been studied to be temperature independent (King and Adamsen, 1992); cellobiase and endoglucanase for cellulose degradation, which were found to be correlated with temperature (Yergeau *et al*., 2007a). In contrast, there were few genes involved in recalcitrant carbon degradation (chitin and lignin) found to be correlated with temperature in this study. For the nitrogen cycle, only 19 genes were correlated with temperature, and most were involved in nitrogen fixation (*nifH*) and denitrification (*nirK* and *nirS*), which was consistent with recent studies that found that *nifH* and *nir* genes were sensitive to temperature (Yergeau *et al*., 2007a; Jung *et al*., 2011).

A total of 165 genes were correlated with pH ($|r| = 0.57$–0.92, $P < 0.01$) (Supporting information Table S6). Interestingly, a majority of these genes were negatively correlated with pH, implying that the microbes hosting these genes preferred the acidic habitats of the donor site (site S). Similar to recent observations that pH had a strong influence on the *copA* genetic structure (Lejon *et al*., 2007), we found a number of *copA* genes to be pH sensitive, most of which were derived from *Proteobacteria*. Three *nirK* genes involved in denitrification were correlated with pH. In contrast, only one *nirS* gene was correlated with pH, although there are similar numbers of *nirK* and *nirS* gene probes on GeoChip. This observation was consistent with recent findings that *nirK*, but not *nirS*, was correlated with pH (Dandie *et al*., 2011).

A striking number of genes, 1163 (14.2% of total detected genes), were correlated with NH_4^+-N ($|r| = 0.56$–0.99, $P < 0.01$), suggesting that there were strong linkages between microbial communities and NH_4^+-N concentrations. Based on the *gyrB* gene, a useful marker of microbial phylogenetic distribution (Hirsch *et al*., 2010; Peeters and Willems, 2011), a number of *Shewanella, Pseudomonas* and *Rhodobacter*-like species of *Proteobacteria* were significantly correlated with NH_4^+-N (data not shown). This was consistent with their important roles in the nitrogen cycle (Chèneby *et al*., 2000; Dandie *et al*., 2007; Gao *et al*., 2009).

Linking microbial genes to soil functional processes

Soil nitrification is mainly a microbially mediated soil functional process. In particular, ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) played important roles in controlling the rates of nitrification (Zhang *et al*., 2011). In this study, the average abundances of both *amoA-AOA* ($r = 0.48$, $P = 0.05$) and *amoA-AOB* ($r = 0.65$, $P = 0.004$) were significantly correlated with nitrification capacity, with a higher correlation obtained for *amoA-AOB* (Fig. 5). However, only four individual *amoA* genes were detected in all samples, including two *amoA-AOA* derived from *Crenarchaeota* and *amoA-AOB* derived from unknown bacteria, which played important roles in controlling nitrification capacity.

We proceeded to examine whether there was a linkage between microbial carbon degradation genes and *in situ* soil CO_2 efflux. However, no significant correlation was detected between the average abundance of microbial carbon degradation genes and soil CO_2 efflux (data not shown), suggesting that other factors played a vital role in determining soil CO_2 efflux.

Discussion

The use of soil transplant as a proxy to study the effects of climate change has been successfully demonstrated in both plant biology and microbiology (Breeuwer *et al*., 2010; De Frenne *et al*., 2011; Vanhala *et al*., 2011). In this study, we showed that 4 years of exposure to colder

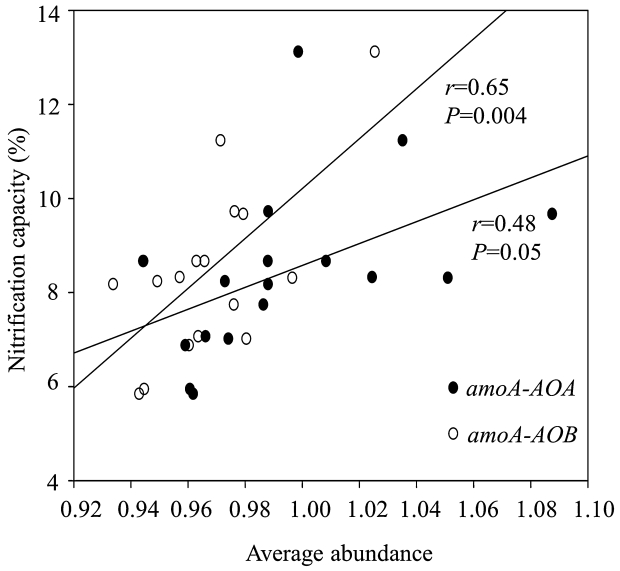

Fig. 5. Pearson correlations between nitrification capacity and the average abundance of *amoA-AOA* and *amoA-AOB*. Filled and open circles represent the average abundance of *amoA-AOA* and *amoA-AOB* respectively.

climates were sufficient to alter soil nitrogen components and microbial communities. The effects of soil transplant were negatively affected by maize cropping. Changes in microbial communities were explainable by the selected environmental attributes, and significant linkages were notable between soil nitrification and related microbial functional groups.

The climatic effect on microbial community composition is under heated debate. It has been shown that soil microbial diversity decreased when latitude increased and was positively correlated with air temperature (Yergeau *et al*., 2007b; Bryant *et al*., 2008). Similarly, a significant latitudinal diversity gradient, with a strong correlation with temperature, was detected in planktonic marine bacteria (Fuhrman *et al*., 2008). In contrast, there were large-scale studies indicating that bacterial diversity was not correlated with temperature or latitude (Fierer and Jackson, 2006; Chu *et al*., 2010; Mi *et al*., 2012). Our observation that the functional diversity of microbial community detected by GeoChip decreased at higher latitudes supported studies showing that soil microbial diversity decreased with the increase of latitude, albeit that the effects of other climate variables (e.g. precipitation and air nitrogen deposition) could not be excluded because of the limited number and size of our study sites. The effects of soil transplant on microbial communities could be attributed to lesser primary productivity, poorer litter quality and colder climate at high latitudes (Ma *et al*., 2004), leading to elevated environmental harshness for microbial communities. However, primary productivity and litter could not be the major causes in this study as the study sites were bare fallow (Supporting information Fig. S1). Climate conditions at the colder site might be the primary cause of changes in microbial community; however, it remained unclear whether changes in microbial communities were mediated by concurrent changes in soil attributes.

By exposing soil to new climate regimes in the transplant experiments, soil inhabitants were pressured to acclimate or adapt to their new environments. The home-site advantage has been well observed in plants (Montalvo and Ellstrand, 2001; Savolainen *et al*., 2007) and also provided an explanation for the observation in our experiments that microbial biomass was reduced by transplant in general. Alternatively, the decrease in microbial biomass might be caused by the transplant procedure. Soil mixing and transportation during transplant might have an adverse effect on microbial communities. To minimize this possibility, we mocked soil transplant for the control site and waited for 4 years so that the transplant effect could subside. The results of bacterial/fungal biomass ratios suggested that the alteration in microbial biomass could be linked to environmental attributes, as there were significant correlations with soil moisture and pH (Fig. 2B). Notably, it has been well established that the dry–wet climatic gradient negatively altered the ratios of bacteria to fungi (Frey *et al*., 1999), which was in line with our finding.

Soil transplant caused alterations in microbial biomass and functional community composition. In contrast, only soil nitrogen components, but not those of carbon, phosphorus or potassium, showed notable sensitivity to soil transplant. These results were consistent with recent findings that soil microbial parameters were sensitive indicators of the change in soil conditions (Lagomarsino *et al*., 2009; Li *et al*., 2009). These soil microbial parameters could replace soil geochemical indicators since carbon, phosphorus and potassium varied slowly over time. Soil microbial status, including microbial biomass (García-Orenes *et al*., 2010), enzyme activity (García-Ruiz *et al*., 2008) and community diversity (Sharma *et al*., 2011), has been tested as an indicator of soil conditions. Here, we show that functional composition of microbial communities, as detected by GeoChip, can potentially be used to indicate the changes in soil conditions.

Although changes in soil pH were not significant, our correlation analyses indicated that it was an important soil attribute in shaping microbial communities. Consistently, soil pH has been shown to impose a strong influence on microbial community abundance and composition (Fierer and Jackson, 2006; Nilsson *et al*., 2007), either directly or indirectly through concurrent environmental attributes such as nutrient availability (e.g. the shift between NH_3 and NH_4^+). At the pH interval of 4–7, it was shown that higher soil pH was correlated with higher bacterial

diversity (Lauber *et al*., 2009; Rousk *et al*., 2010), making it a good predictor of bacterial community composition. In contrast, fungi tolerated wider pH ranges than bacteria, and their diversity was only weakly linked to pH (Nevarez *et al*., 2009; Rousk *et al*., 2010). The differential sensitivity of bacteria and fungi to pH was also demonstrated in our finding that bacterial/fungal biomass ratios were positively correlated with soil pH (Fig. 2B).

Maize cropping dampened the effect of soil transplant on microbial communities (Supporting information Fig. S4), suggesting that it played a major role in shaping microbial communities. Cropping can influence microbial communities through a number of intertwined mechanisms. As a result of the increased aboveground litter input to microbes, cropping has been shown to significantly increase microbial diversity (Zhang *et al*., 2005). Meanwhile, cropping directly impacts the community composition of root-associated organisms or indirectly impacts the community composition of root-associated organisms by root exudates (Wardle *et al*., 2004). Therefore, cropping could interact with climate change-induced effects by enabling soil microbial communities to be more resistant to climate change (Potthoff *et al*., 2006).

amoA-AOA and *amoA-AOB* were correlated with soil nitrification capacity in our experiments, raising the possibility that DNA abundance can be used to indicate rates of soil ecoprocesses such as greenhouse gas emission. However, it has been commonly assumed that abundance of microbial community DNA measures the metabolic potential, and only abundance of messenger RNA (mRNA) or protein represents functional activity. Unfortunately, the detection of soil mRNA from field samples imposes a number of challenges including interference from ribosomal RNA (rRNA) and transfer RNA (tRNA), strict transport and storage requirements, rapid turnover and severe instability (Sessitsch *et al*., 2002; Mettel *et al*., 2010), rendering the profiling and quantification of mRNA very difficult, if not intractable, in many samples. In contrast, DNA profiling and quantification are substantially reliable compared with mRNA. We argue that changes in DNA can be used to estimate functional activity because it indicates that the microbial population is active across the samples, thus providing a good alternative to short-lived RNAs in estimating functional activity. In support of this viewpoint, DNA abundance of *nirS–nosZ* genes was recently shown to correlate with N_2O emission in soils, suggesting that abundance of these functional genes could serve as a proxy for greenhouse gas (N_2O) emissions (Morales *et al*., 2010). Similarly, other studies also demonstrated that DNA abundance could be used for assessing the activities of microbial communities (Van Nostrand *et al*., 2011; Liang *et al*., 2012).

Recognizing the effects of environmental disturbance on soil microbial community is crucial for microbial resource conservation. Our results showed that microbial communities were significantly altered by soil transplant into colder regions, but the effects of soil transplant were dampened by maize cropping. Their interactive impacts reshaped soil microbial communities and played an important role in dictating the microbial response. Our results illustrate complex changes in terrestrial microbial ecosystems under different climate change scenarios and thus highlight the need to further understand how microbial communities will potentially be affected and their feedbacks to climate change.

Experimental procedures

Site description and sampling

The transplant experiments were carried out in agricultural experimental stations of the Chinese Academy of Sciences at three sites, from Yingtan, Jiangxi Province of southern China to Fengqiu, Henan Province of central China and Hailun, Heilongjiang Province of northern China, across different climatic regimes from subtropical to warm temperate and cold temperate climate respectively. Soil at site S was characterized as red soil derived from quaternary red clay, which is representative of about 13.39% of soil in China. At each site, triplicate soil plots were established with and without maize cropping. In October 2005, soil from site S ($1.2 \times 1.4 \times 1.0$ m soil) was removed in five layers with a depth of 0.2 m each layer. Soil from each layer was mixed, transported to sites SC and SN and placed into soil holes of the same size according to the original sequence. The plots at site S were mock transplanted but remained in place to serve as controls. The transplanted and control plots were separated from the surrounding environment by 20 cm brick walls. The plots were paved underneath with quartz sand. The inner walls were covered with waterproof membrane to prevent infiltration. Moreover, the plots were built to be 30 cm above the ground to prevent invasion by surrounding soil. Maize was planted in 2006 at all three sites. Neither fertilization nor irrigation was managed. Maize was harvested and sowed once a year. The samples with maize cropping from these three sites were designated as Sm, SCm and SNm. For bare fallow plots, no management was undertaken except for manual de-weeding, whenever weeds were spotted, to prevent plant biomass or litter input.

Soils from the three study sites were sampled in August–September, 2009. Ten soil cores were composited from surface soil (0–20 cm) within each plot and sealed in a polythene wrapper, then stored on ice and transported to the laboratory. Any visible living plant material (e.g. roots) was manually removed from the composited soil in the lab. The soil was then divided into two subsamples and stored at either 4°C for soil attribute measurements or –80°C for PLFAs and GeoChip analysis.

Soil geochemical attributes, vegetation and functional processes

All of the measurements of soil geochemical attributes were completed within 2 months after sampling. Soil moisture

content was measured gravimetrically with 10 g fresh soil by incubating in an oven at 105°C for 12 h. To determine nitrate (NO_3^--N) and ammonium (NH_4^+-N) concentrations, 20 g fresh soil was suspended in 100 ml of 1 mol L^{-1} KCL for 1 h with gentle shaking. NO_3^--N and NH_4^+-N concentrations were measured in the suspension by Auto Analyser 3 (Bran + Luebbe GmbH, Germany). Subsamples of soil for the analysis of other geochemical attributes were air dried and kept at room temperature. Soil pH was measured using a glass electrode (Mettler Toledo Instruments, Shanghai, China) with a ratio of 1:2.5 of soil (10 g, sieved to 1 mm mesh) to water. Soil organic matter was determined by dichromate oxidation with external heat and titration with ferrous ammonium sulphate (Mebius, 1960). Soil TN was determined by semimicro-Kjeldahl digestion (Page, 1982). Soil AN was measured using the Illinois Soil Nitrogen Test diffusion method (Khan *et al*., 2001). Soil TP was determined colorimetrically using the molybdate method (Murphy and Riley, 1962). Soil available phosphorus (AP) was determined colorimetrically based on the Olsen method (Olsen *et al*., 1954). Soil TK was extracted by incubation with sodium hydroxide, and soil available potassium (AK) was extracted by incubation with 1.0 mol L^{-1} ammonium acetate for 0.5 h followed by filtration. The concentrations in the extracts were determined by flame photometry (CANY Precision Instrument, Shanghai, China). Climate attributes, including annual average temperature, rainfall and relative humidity were obtained from the experimental stations' meteorological observation data.

When sampling, all the crops in one plot were harvested first, after which stalks were selected and immediately dried in the oven until the weight remained constant. Based on the water content in the stalks, aboveground biomass and grain weight were calculated for each plot.

Soil temperature and CO_2 efflux at 0–5 cm were measured using a soil respiration chamber (LI-6400-09; LiCor, Lincoln, NE, USA) connected to a portable photosynthesis system (LI-6400) at the time of sampling. To measure soil nitrification potential, soil (70 g; oven dry weight equivalent) was placed in a pre-weighed beaker, then 31.5 mg $(NH_4)_2SO_4$ and additional water were added to adjust the final soil moisture content to be 70% of water holding capacity. The beakers were capped with perforated Parafilm, mixed well and incubated at 25°C for 14 days. Samples were monitored to maintain constant moisture content. At days 0, 2, 3, 7, 10 and 13, the concentrations of NH_4^+, NO_2^- and NO_3^- were measured by a continuous flow analyser (Bran + Luebbe GmbH Inc., Hamburg, Germany).

PLFA experiments

Phospholipid fatty acids were extracted using a modified procedure as previously described (Brant *et al*., 2006). Briefly, 2 g of soil for each sample was incubated in an extraction mixture containing methanol, chloroform and phosphate buffer (2:1:0.8, v/v/v) for 2 h. The lipids were separated into neutral lipids, glycolipids and phospholipids using silica acid columns, then phospholipids were methylated to fatty-acid methyl esters and analysed using a Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA). The PLFAs i14:0, i15:0, a15:0, 15:0, i16:0, 16:1w5c, i17:0, a17:0, cy17:0, 17:0, 16:1w9c, 18:1w5c, 18:1w7c and cy19:0 were chosen as bacterial markers (Frostegård and Bååth, 1996); while 18:1w9c was chosen to represent fungi (Mikola and Setälä, 1999). In addition, 10Me17:0, 10Me18:0 were used as markers for actinomycetes (Frostegård *et al*., 1993).

GeoChip experiments

Deoxyribonucleic acid for GeoChip analysis was extracted from 5 g of well mixed soil by a freeze-grinding mechanical lysis method as previously described (Zhou *et al*., 1996). Deoxyribonucleic acid was purified by electrophoresis with low melting point agarose gel, followed by extraction with phenol–chloroform prior to butanol precipitation. The final DNA concentrations were quantified with a PicoGreen kit. Deoxyribonucleic acid quality was measured by a Nanodrop (NanoDrop Technologies Inc., Wilmington, DE, USA), and the values of 260/280 nm of 1.8, and 260/230 nm of over 1.5 were considered acceptable.

Purified DNA (2 μg) was labelled with Cy-5 fluorescent dye using random primers, then purified and dried at 45°C for 45 min (SpeedVac, ThermoSavant, Milford, MA, USA), prior to suspension in a hybridization buffer [40% formamide, 3 × SSC, 10 μg of unlabelled herring sperm DNA (Promega, Madison, WI) and 0.1% SDS]. Labelled DNA was hybridized with GeoChip 3.0 at 42°C for 12 h. After washing with 1 × SSC–0.2% SDS and 0.1 × SSC–0.2% SDS for 5 min and 0.1 × SSC for 30 s, microarrays were scanned (ProScan Array, Perkin Elmer, Waltham, MA, USA), and the signal intensity for each probe was measured by using IMAGENE software (6.0 premium version, Biodiscovery, El Segundo, CA, USA) (He *et al*., 2010).

GeoChip data normalization and analysis

Data normalization and analysis were conducted as previously described (Yang *et al*., 2013; 2014). Raw data was downloaded from the website (http://ieg.ou.edu/microarray/) and analysed according to the following steps: (i) poor-quality spots flagged as 1 or 3 and with SNR less than 2.0 were removed, (ii) after removing bad spots, data normalization of each spot was performed by logarithm-transforming each signal intensity and then dividing the transformed signal intensity by the mean intensity across all genes on the microarray, and (iii) probes detected in only one replicate were eliminated as noise.

STATISTICAL ANALYSIS SYSTEM software (SAS Institute, Cary, NC, USA) was used to examine the differences in environmental attributes based on one-way analysis of variance (ANOVA) at all three sites. Using an online pipeline (http://ieg.ou.edu/), permutational multivariate analysis of variance (Adonis) based on the Bray–Curtis index to calculate distance matrices was used to examine the treatment effects on functional (GeoChip) compositions of soil microbial communities. Detrended correspondence analysis and hierarchical cluster analysis were performed with the Vegan package in R v.2.12.2 (R Development Core Team, 2011) to illustrate the differences in soil microbial community composition among samples. Mantel tests were performed based on Bray–Curtis and Euclidean indexes to test the correlations between soil attributes and soil microbial community composition.

Canonical correspondence analysis was performed using Vegan package in R v.2.12.2. The selected attributes in canonical correspondence analysis (CCA) modelling were based on VIFs, which stepwise removed redundant attributes, resulting in six environmental attributes (annual average temperature, pH, relative humidity, AN, NH_4^+-N and aboveground biomass) with VIFs less than 20. Pearson correlation coefficients were calculated to determine correlations between functional genes and selected soil attributes or soil ecoprocesses. Also, Pearson correlations between soil and climate attributes were calculated. Significant differences between treatments and control were analysed by two-tailed *t*-tests based on two-sample equal variance. The significance of interactive effects of soil transplant and maize cropping was tested by two-way ANOVA in SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

Acknowledgements

The authors thank Hailun, Fengqiu and Yingtan Research Station staff for sampling assistance and the anonymous reviewers for constructive comments and suggestions for the improvement of this manuscript. This research was supported by grants to Yunfeng Yang from the National Natural Science Foundation of China (41171201) and State Key Joint Laboratory of Environment Simulation and Pollution Control (11Z03ESPCT); grants to Bo Sun from National Basic Research Program of China (2011CB100506) and the National Natural Science Foundation of China (41271258); and grants to Jizhong Zhou from the United States Department of Energy, Biological Systems Research on the Role of Microbial Communities in C Cycling Program (DE-SC0004601), the U.S. National Science Foundation under the contract (NSF EF-1065844) and by the United States Department of Agriculture (Project 2007–35319-18305) through NSF-USDA Microbial Observatories Program. This study made use of GeoChip and associated computational pipelines whose development was funded by ENIGMA – Ecosystems and Networks Integrated with Genes and Molecular Assemblies through the Office of Science, Office of Biological and Environmental Research, the US Department of Energy under Contract No. DE-AC02-05CH11231.

Preliminary data accessibility section

All of the GeoChip data will be accessible from GEO database (http://www.ncbi.nlm.nih.gov/geo/). The accessible number is GSE51592.

References

Alley, R.B., Marotzke, J., Nordhaus, W., Overpeck, J., Peteet, D., Pielke, R., *et al*. (2003) Abrupt climate change. *Science* **299:** 2005–2010.

Brant, J.B., Sulzman, E.W., and Myrold, D.D. (2006) Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. *Soil Biol Biochem* **38:** 2219–2232.

Breeuwer, A., Heijmans, M.P.D., Robroek, B.M., and Berendse, F. (2010) Field simulation of global change: transplanting Northern Bog Mesocosms Southward. *Ecosystems* **13:** 712–726.

Bryant, J.A., Lamanna, C., Morlon, H., Kerkhoff, A.J., Enquist, B.J., and Green, J.L. (2008) Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proc Natl Acad Sci* **105:** 11505–11511.

Chèneby, D., Philippot, L., Hartmann, A., Hénault, C., and Germon, J.-C. (2000) 16S rDNA analysis for characterization of denitrifying bacteria isolated from three agricultural soils. *FEMS Microbiol Ecol* **34:** 121–128.

Chu, H., Fierer, N., Lauber, C.L., Caporaso, J., Knight, R., and Grogan, P. (2010) Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. *Environ Microbiol* **12:** 2998–3006.

Dandie, C.E., Burton, D.L., Zebarth, B.J., Trevors, J.T., and Goyer, C. (2007) Analysis of denitrification genes and comparison of nosZ, cnorB and 16S rDNA from culturable denitrifying bacteria in potato cropping systems. *Syst Appl Microbiol* **30:** 128–138.

Dandie, C.E., Wertz, S., Leclair, C.L., Goyer, C., Burton, D.L., Patten, C.L., *et al*. (2011) Abundance, diversity and functional gene expression of denitrifier communities in adjacent riparian and agricultural zones. *FEMS Microbiol Ecol* **77:** 69–82.

De Frenne, P., Brunet, J., Shevtsova, A., Kolb, A., Graae, B.J., Chabrerie, O., *et al*. (2011) Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. *Glob Change Biol* **17:** 3240–3253.

Falkowski, P.G., Fenchel, T., and Delong, E.F. (2008) The microbial engines that drive Earth's biogeochemical cycles. *Science* **320:** 1034–1039.

Fierer, N., and Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* **103:** 626–631.

Frey, S.D., Elliott, E.T., and Paustian, K. (1999) Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. *Soil Biol Biochem* **31:** 573–585.

Frostegård, A., and Bååth, E. (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol Fertil Soils* **22:** 59–65.

Frostegård, Å., Bååth, E., and Tunlio, A. (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biol Biochem* **25:** 723–730.

Fuhrman, J.A., Steele, J.A., Hewson, I., Schwalbach, M.S., Brown, M.V., Green, J.L., and Brown, J.H. (2008) A latitudinal diversity gradient in planktonic marine bacteria. *Proc Natl Acad Sci USA* **105:** 7774–7778.

Gao, H., Yang, Z.K., Barua, S., Reed, S.B., Romine, M.F., Nealson, K.H., *et al*. (2009) Reduction of nitrate in Shewanella oneidensis depends on atypical NAP and NRF systems with NapB as a preferred electron transport protein from CymA to NapA. *ISME J* **3:** 966–976.

García-Orenes, F., Guerrero, C., Roldán, A., Mataix-Solera, J., Cerdà, A., Campoy, M., *et al*. (2010) Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. *Soil Tillage Res* **109:** 110–115.

García-Ruiz, R., Ochoa, V., Hinojosa, M.B., and Carreira, J.A. (2008) Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. *Soil Biol Biochem* **40:** 2137–2145.

Hansen, J., Sato, M., Ruedy, R., Lo, K., Lea, D.W., and Medina-Elizalde, M. (2006) Global temperature change. *PNAS* **103:** 14288–14293.

Hare, S.R., and Mantua, N.J. (2000) Empirical evidence for North Pacific regime shifts in 1977 and 1989. *Prog Oceanogr* **47:** 103–145.

He, Z., Deng, Y., Van Nostrand, J.D., Tu, Q., Xu, M., Hemme, C.L., *et al*. (2010) GeoChip 3.0 as a high-throughput tool for analyzing microbial community composition, structure and functional activity. *ISME J* **4:** 1167–1179.

Hirsch, P.R., Mauchline, T.H., and Clark, I.M. (2010) Culture-independent molecular techniques for soil microbial ecology. *Soil Biol Biochem* **42:** 878–887.

Jung, J., Yeom, J., Kim, J., Han, J., Lim, H.S., Park, H., *et al*. (2011) Change in gene abundance in the nitrogen biogeochemical cycle with temperature and nitrogen addition in Antarctic soils. *Res Microbiol* **162:** 1018–1026.

Khan, S.A., Mulvaney, R.L., and Hoeft, R.G. (2001) A simple soil test for detecting sites that are nonresponsive to nitrogen fertilization this study was a part of Project ILLU-65-0326, Illinois Agric. Exp. Stn. *Soil Sci Soc Am J* **65:** 1751–1760.

King, G.M., and Adamsen, A.P.S. (1992) Effects of temperature on methane consumption in a forest soil and in pure cultures of the Methanotroph Methylomonas rubra. *Appl Environ Microbiol* **58:** 2758–2763.

Lagomarsino, A., Moscatelli, M.C., Di Tizio, A., Mancinelli, R., Grego, S., and Marinari, S. (2009) Soil biochemical indicators as a tool to assess the short-term impact of agricultural management on changes in organic C in a Mediterranean environment. *Ecol Indic* **9:** 518–527.

Lauber, C.L., Hamady, M., Knight, R., and Fierer, N. (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* **75:** 5111–5120.

Lejon, D.P.H., Nowak, V., Bouko, S., Pascault, N., Mougel, C., Martins, J.M.F., and Ranjard, L. (2007) Fingerprinting and diversity of bacterial copA genes in response to soil types, soil organic status and copper contamination. *FEMS Microbiol Ecol* **61:** 424–437.

Li, Y.-T., Rouland, C., Benedetti, M., Li, F., Pando, A., Lavelle, P., and Dai, J. (2009) Microbial biomass, enzyme and mineralization activity in relation to soil organic C, N and P turnover influenced by acid metal stress. *Soil Biol Biochem* **41:** 969–977.

Liang, Y., Van Nostrand, J.D., N'Guessan, L.A., Peacock, A.D., Deng, Y., Long, P.E., *et al*. (2012) Microbial functional gene diversity with a shift of subsurface redox conditions during in situ uranium reduction. *Appl Environ Microbiol* **78:** 2966–2972.

Ma, X., Chen, T., Zhang, G., and Wang, R. (2004) Microbial community structure along an altitude gradient in three different localities. *Folia Microbiol* **49:** 105–111.

McAnena, A., Flögel, S., Hofmann, P., Herrle, J., Griesand, A., Pross, J., *et al*. (2013) Atlantic cooling associated with a marine biotic crisis during the mid-Cretaceous period. *Nat Geosci* **6:** 558–561.

Mebius, L.J. (1960) A rapid method for the determination of organic carbon in soil. *Anal Chim Acta* **22:** 120–124.

Mettel, C., Kim, Y., Shrestha, P.M., and Liesack, W. (2010) Extraction of mRNA from soil. *Appl Environ Microbiol* **76:** 5995–6000.

Mi, L., Wang, G., Jin, J., Sui, Y., Liu, J., and Liu, X. (2012) Comparison of microbial community structures in four Black soils along a climatic gradient in northeast China. *Can J Soil Sci* **92:** 543–549.

Mikola, J., and Setälä, H. (1999) Interplay of omnivory, energy channels and C availability in a microbial-based soil food web. *Biol Fertil Soils* **28:** 212–218.

Montalvo, A.M., and Ellstrand, N.C. (2001) Transplantation of the subshrub lotus scoparius: testing the home-site advantage hypothesis. *Conserv Biol* **14:** 1034–1045.

Morales, S.E., Cosart, T., and Holben, W.E. (2010) Bacterial gene abundances as indicators of greenhouse gas emission in soils. *ISME J* **4:** 799–808.

Murphy, J., and Riley, J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* **27:** 31–36.

Nevarez, L., Vasseur, V., Le Madec, A., Le Bras, M., Coroller, L., Leguérinel, I., and Barbier, G. (2009) Physiological traits of *Penicillium glabrum* strain LCP 08.5568, a filamentous fungus isolated from bottled aromatised mineral water. *Int J Food Microbiol* **130:** 166–171.

Nilsson, L.O., Bååth, E., Falkengren-Grerup, U., and Wallander, H. (2007) Growth of ectomycorrhizal mycelia and composition of soil microbial communities in oak forest soils along a nitrogen deposition gradient. *Oecologia* **153:** 375–384.

Olsen, S.R., Cole, C., and Watanabe, F.S. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture Circular No. 939. Banderis, A. D., Barter, D. H., and Anderson, K. Agricultural and Advisor.

Page, A.L. (1982) *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties: American Society of Agronomy*. Madison, WI, USA: Soil Science Society of America.

Peeters, K., and Willems, A. (2011) The *gyrB* gene is a useful phylogenetic marker for exploring the diversity of Flavobacterium strains isolated from terrestrial and aquatic habitats in Antarctica. *FEMS Microbiol Lett* **321:** 130–140.

Petchey, O.L., McPhearson, P.T., Casey, T.M., and Morin, P.J. (1999) Environmental warming alters food-web structure and ecosystem function. *Nature* **402:** 69–72.

Potthoff, M., Steenwerth, K.L., Jackson, L.E., Drenovsky, R.E., Scow, K.M., and Joergensen, R.G. (2006) Soil microbial community composition as affected by restoration practices in California grassland. *Soil Biol Biochem* **38:** 1851–1860.

R Development Core Team (2011) *R: A Language and Environmental for Statistical Computing*. Vienna, Austria: R Foundation for statistical computing.

Reed, H.E., and Martiny, J.B.H. (2007) Testing the functional significance of microbial composition in natural communities. *FEMS Microbiol Ecol* **62:** 161–170.

Rinnan, R., Michelsen, A., Bååth, E., and Jonasson, S. (2007) Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. *Glob Change Biol* **13:** 28–39.

Roesch, L.F.W., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D., *et al*. (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME J* **1:** 283–290.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., *et al*. (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* **4:** 1340–1351.

Rowe, E.C., Emmett, B.A., Frogbrook, Z.L., Robinson, D.A., and Hughes, S. (2012) Nitrogen deposition and climate effects on soil nitrogen availability: influences of habitat type and soil characteristics. *Sci Total Environ* **434:** 62–70.

Savolainen, O., Pyhäjärvi, T., and Knürr, T. (2007) Gene flow and local adaptation in trees. *Annu Rev Ecol Evol Syst* **38:** 595–619.

Sessitsch, A., Gyamfi, S., Stralis-Pavese, N., Weilharter, A., and Pfeifer, U. (2002) RNA isolation from soil for bacterial community and functional analysis: evaluation of different extraction and soil conservation protocols. *J Microbiol Methods* **51:** 171–179.

Sharma, S.K., Ramesh, A., Sharma, M.P., Joshi, O.P., Govaerts, B., Steenwerth, K.L., and Karlen, D.L. (2011) Microbial community structure and diversity as indicators for evaluating soil quality. In *Biodiversity, Biofuels, Agroforestry and Conservation Agriculture*. Lichtfouse, E. (ed.). Netherlands: Springer, pp. 317–358.

Shaw, M.R., and Harte, J. (2001) Response of nitrogen cycling to simulated climate change: differential responses along a subalpine ecotone. *Glob Change Biol* **7:** 193–210.

Van Nostrand, J.D., Wu, L., Wu, W.-M., Huang, Z., Gentry, T.J., Deng, Y., *et al*. (2011) Dynamics of microbial community composition and function during in situ bioremediation of a uranium-contaminated aquifer. *Appl Environ Microbiol* **77:** 3860–3869.

Vanhala, P., Karhu, K., Tuomi, M., Björklöf, K., Fritze, H., HyvÄRinen, H., and Liski, J. (2011) Transplantation of organic surface horizons of boreal soils into warmer regions alters microbiology but not the temperature sensitivity of decomposition. *Glob Change Biol* **17:** 538–550.

Walther, G.R. (2010) Community and ecosystem responses to recent climate change. *Philos Trans R Soc B Biol Sci* **365:** 2019–2024.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., and Wall, D.H. (2004) Ecological linkages between aboveground and belowground biota. *Science* **304:** 1629–1633.

Yang, Y., Wu, L., Lin, Q., Yuan, M., Xu, D., Yu, H., *et al*. (2013) Responses of the functional structure of soil microbial community to livestock grazing in the Tibetan alpine grassland. *Glob Change Biol* **19:** 637–648.

Yang, Y., Gao, Y., Wang, S., Xu, D., Yu, H., Wu, L., *et al*. (2014) The microbial gene diversity along an elevation gradient of the Tibetan grassland. *ISME J* **8:** 430–440.

Yergeau, E., Kang, S., He, Z., Zhou, J., and Kowalchuk, G.A. (2007a) Functional microarray analysis of nitrogen and carbon cycling genes across an Antarctic latitudinal transect. *ISME J* **1:** 163–179.

Yergeau, E., Bokhorst, S., Huiskes, A.H.L., Boschker, H.T.S., Aerts, R., and Kowalchuk, G.A. (2007b) Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. *FEMS Microbiol Ecol* **59:** 436–451.

Zhang, L.-M., Hu, H.-W., Shen, J.-P., and He, J.-Z. (2011) Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. *ISME J* **6:** 1032–1045.

Zhang, W., Parker, K.M., Luo, Y., Wan, S., Wallace, L.L., and Hu, S. (2005) Soil microbial responses to experimental warming and clipping in a tallgrass prairie. *Glob Change Biol* **11:** 266–277.

Zhou, J., Bruns, M.A., and Tiedje, J.M. (1996) DNA recovery from soils of diverse composition. *Appl Environ Microbiol* **62:** 316–322.

Zhou, J., Xue, K., Xie, J., Deng, Y., Wu, L., Cheng, X., *et al*. (2012) Microbial mediation of carbon-cycle feedbacks to climate warming. *Nat Clim Chang* **2:** 106–110.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. The effect of soil transplant on microbial alpha-diversity in bare fallow samples. Alpha-diversity was calculated by Simpson index. S: soil samples in southern China (Yintan); SC: soil samples transplanted from southern China to central China (Fengqiu); SN: soil samples transplanted from southern China to northern China (Hailun). Data are presented as mean ± standard errors. Significant differences between S and SC or between S and SN were analysed by two-tailed *t*-tests with two-sample equal variance and indicated by '*' for $P < 0.1$ and '**' for $P < 0.05$ respectively.

Fig. S2. Comparison of microbial group biomass in PLFA experiments for bare fallow samples. Data are presented as mean ± standard errors. Significant differences between S and SC or between S and SN were analysed by two-tailed *t*-tests with two-sample equal variance and indicated by '**' for $P < 0.05$. Symbols as in Supporting information Fig. S1.

Fig. S3. Detrended correspondence analysis of soil microbial communities for all of soil samples based on GeoChip data. Symbols as in Supporting information Fig. S1; m: maize cropping.

Fig. S4. Observed versus additive effects of soil transplant and maize cropping on microbial alpha-diversities. The observed effect = SCm (or SNm) − S. The additive effect = SC (or SN) − S + Sm − S. Significant interactive effects were indicated by '***' for $P < 0.01$. Symbols as in Supporting information Fig. S3.

Table S1. Comparison of gene category distribution at different sites.

Table S2. Genes detected only at site S[a], and at neither sites SC nor SN.

Table S3. The statistical test of Adonis[a] to analyse the effects of dual factors of soil transplant and maize cropping on soil microbial community compositions measured by GeoChip.

Table S4. Environmental attributes of samples with maize cropping.

Table S5. Genes significantly ($P < 0.01$) correlated with annual average temperature.

Table S6. Genes significantly correlated ($P < 0.01$) with pH.