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Microbial mediation of carbon-cycle feedbacks to climate warming

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Understanding the mechanisms of biospheric feedbacks to climate change is critical to project future climate warming[1–3]. Although microorganisms catalyse most biosphere processes related to fluxes of greenhouse gases, little is known about the microbial role in regulating future climate change[4]. Integrated metagenomic and functional analyses of a long-term warming experiment in a grassland ecosystem showed that microorganisms play crucial roles in regulating soil carbon dynamics through three primary feedback mechanisms: shifting microbial community composition, which most likely led to the reduced temperature sensitivity of heterotrophic soil respiration; differentially stimulating genes for degrading labile but not recalcitrant carbon so as to maintain long-term soil carbon stability and storage; and enhancing nutrient-cycling processes to promote plant nutrient-use efficiency and hence plant growth. Elucidating microbially mediated feedbacks is fundamental to understanding ecosystem responses to climate warming and provides a mechanistic basis for carbon–climate modelling.

Feedback between terrestrial carbon (C) and climate warming is one of the major uncertainties in projecting future climate warming[5,6]. Most carbon–climate modelling studies predict a positive feedback in that warming leads to a decrease in ecosystem C storage due to a warming-induced increase in soil C release through respiration[1,7,8]. Results from various experimental studies on the effects of climate warming on ecosystem C storage, however, are controversial and contradictory[9]. Such controversy is partially due to the lack of a mechanistic understanding of the feedback responses of below-ground microbial communities to climate warming[1–4,10] because most of those experiments have primarily focused on plant communities.

Although microorganisms mediate biogeochemical cycles of C, nitrogen (N), phosphorus (P), sulphur (S) and various metals, and play critical roles in ecosystem C dynamics, their responses and feedback mechanisms to climate warming are poorly understood[4,11]. The lack of a mechanistic understanding of microbial responses is mostly because of technological limitations for analysing microbial communities. The recently emerged metagenomic technologies, such as high-throughput sequencing[12] and GeoChip[13–16], have revolutionized microbial research, allowing us to address research questions previously unapproachable. Here, we used integrated metagenomics technologies to determine the feedback responses of microbial community structure and functions to climate warming in a tall-grass prairie ecosystem in the US Great Plains in Central Oklahoma (34°59′ N, 97°31′ W). On the basis of our previous studies related to above-ground plant community dynamics and ecosystem processes[9,17], we predicted that warming would lead to a reduced temperature sensitivity of respiratory responses through microbial acclimation by shifting microbial community structure rather than through substrate depletion. We also reasoned that different microbial populations would have differential responses to climate warming and that warming would greatly stimulate the functional genes involved in nutrient-cycling processes.

The experimental plots have been subjected to continuous 2 °C warming since 1999 (ref. 17). The C_4 plant biomass in 2007 was significantly higher ($P = 0.01$) under warming but C_3 plant biomass remained unchanged (Fig. 1a), resulting in a plant community shift towards more C_4 species. As a result, $\delta^{13}C$ was significantly higher ($P = 0.03$) under warming than the control (Fig. 2c). Further analysis revealed that, overall, warming increased the input of C_4-derived C by 11.6%, but inputs varied (4.4–16.4%) considerably among three soil fractions across various aggregate sizes (53–250 μm, 250–2,000 μm and >2,000 μm; Supplementary Table S1). In addition, on the basis of a one-tailed paired *t*-test, warming significantly or marginally significantly increased litter input to soil ($P = 0.05$) and below-ground net primary production (BNPP; $P = 0.10$; Fig. 1a). Although warming stimulated C input into soils, it also significantly stimulated soil respiration ($P = 0.008$; Fig. 1f), which is the second largest C flux between terrestrial ecosystems and the atmosphere in the global C cycle[18,19]. It seems that the increases in C loss through soil respiration were approximately offset by the increased net primary production[9]. As a result, the total soil organic C, as well as the labile and recalcitrant C, remained unchanged (Fig. 1b). Furthermore, the soil moisture content and pH in 2008 were not affected by warming.

Three microbially mediated responses and feedback processes seem to play significant roles in maintaining the balance of soil C under warming. First, the changes in above-ground plant composition, biomass and the soil physiochemical environment[19] markedly altered the composition and structure of microbial communities. Less than half of the functional genes and operational taxonomic units detected overlapped between warming and the control (Supplementary Information), but no difference in the phylogenetic/functional gene richness and diversity was observed between warming and control plots (Supplementary Table S2). Furthermore, warming markedly shifted both the functional and

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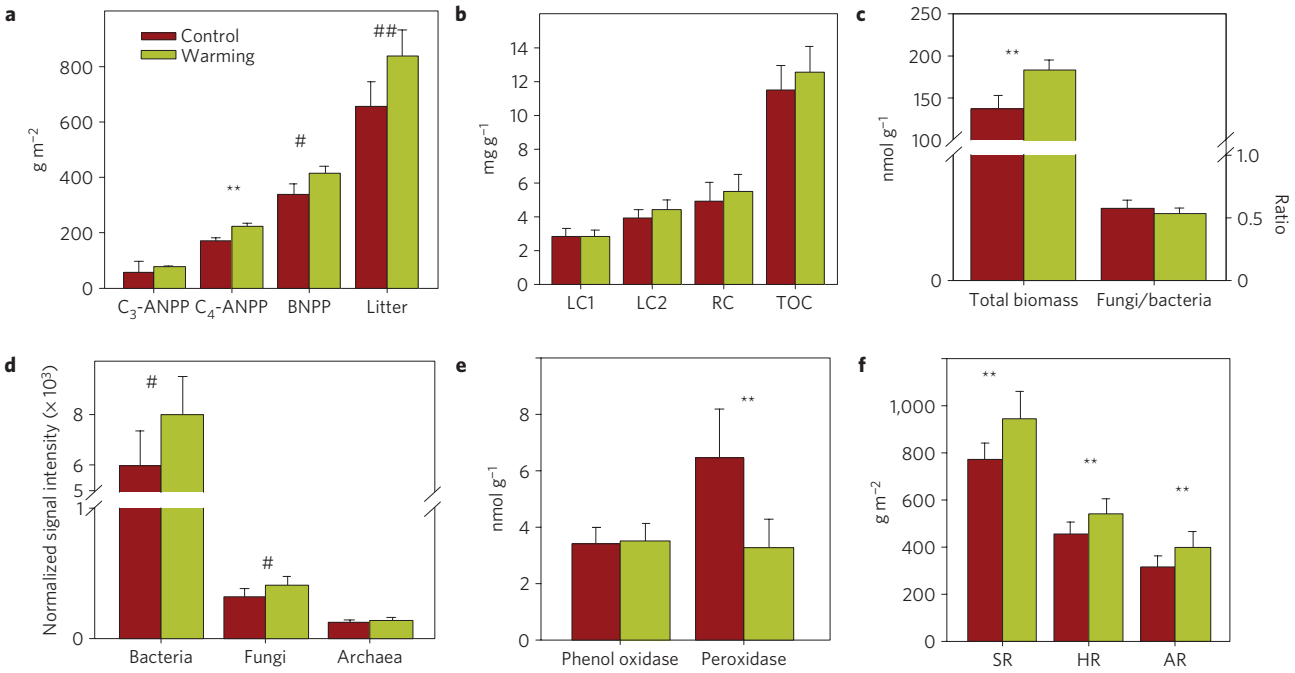

Figure 1 | Effects of warming on a series of plant and soil variables. a–f, Effects of warming on ANPP from C_3 and C_4 species, BNPP and litter biomass (**a**); soil C pools, including total organic C (TOC), labile-C pool 1 (LC1) and 2 (LC2) and recalcitrant C (RC; **b**); total PLFAs for soil microbial biomass and the ratio of fungi to bacterial biomass calculated by the signature PLFAs for fungi and bacteria (**c**); abundance of detected genes belonging to bacteria, fungi and archaea by GeoChip (**d**); enzyme activity of phenol oxidase and peroxidase (**e**); and *in situ* annual soil respiration (SR), heterotrophic (HR) and autotrophic respiration (AR) measured (**f**). Error bars indicate standard error of the mean ($n = 18$ for phenol oxidase and peroxidase enzyme activities as three technical replicates were employed, and $n = 6$ for the rest of the variables). The differences between warming and the control were tested by two-tailed paired *t*-tests, indicated by *** when $P < 0.01$, ** when $P < 0.05$ or * when $P < 0.10$. The differences for some parameters were also tested with one-tailed paired *t*-tests as indicated by ## when $P < 0.05$ and # when $P < 0.10$.

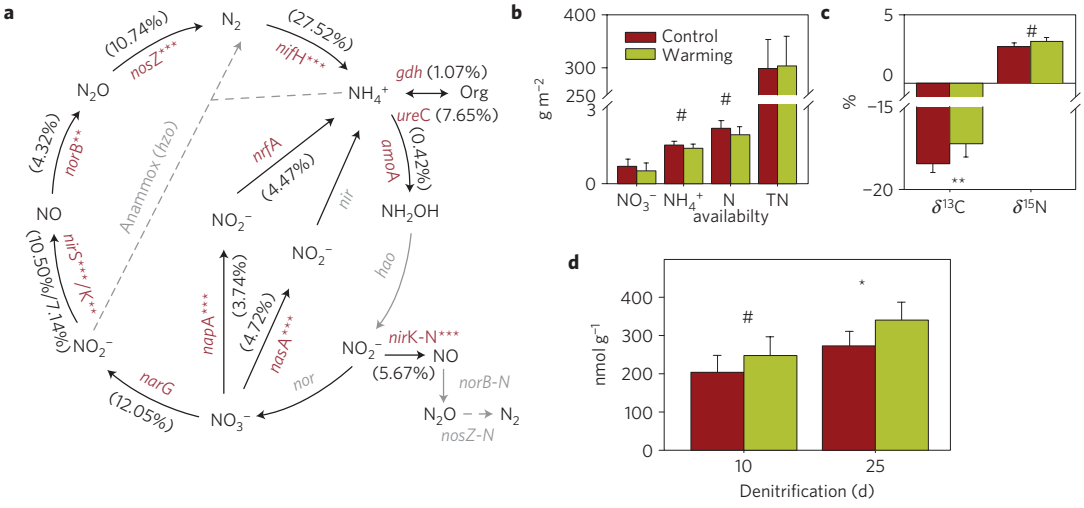

Figure 2 | Impacts of warming on N cycling. a, The relative changes of detected N-cycling genes under warming. The percentage in brackets for each gene is calculated by dividing the total signal intensities of each gene by the total signal intensities of all detected N-cycling genes, and then weighted by the fold change (warming/control) of each gene. Red-coloured genes had higher signal intensities under warming than the control; grey-coloured genes were not present on the version of GeoChip used, or were undetected in those samples. **b**, Soil nitrate (NO_3^-), ammonia (NH_4^+), N availability and total N (TN). **c**, $\delta^{13}C$ and $\delta^{15}N$ values (‰) for whole soils. **d**, Denitrification potential measured at 10 and 25 days. Error bars represent standard error ($n = 5$ for denitrification measured at 25 days because of missing data, and $n = 6$ for the rest of the variables). The differences of most parameters between warming and the control were tested by two-tailed paired *t*-tests (*), as well as one-tailed paired *t*-tests (#) for a few parameters (see Fig. 1 caption).

phylogenetic structures of microbial communities, as indicated by detrended correspondence analysis (DCA)-based ordination for both GeoChip (Supplementary Fig. S1) and pyrosequencing (Supplementary Fig. S2) data, respectively. Three complimentary non-parametric multivariate statistical tests (ANOISM, adonis and MRPP) revealed that the functional and phylogenetic community structures were significantly different between the warming and control treatments (Table 1). In addition, canonical correspondence analysis (CCA) showed that microbial community functional composition and structure were significantly ($F = 1.190$, $P = 0.017$)

Table 1 | Significance tests of the effects of warming on the overall microbial community structure with three different statistical approaches.

Data sets	adonis*		ANOSIM†		MRPP‡	
	F	*P* §	*R*	*P* §	*δ*	*P* §
Functional genes	0.138	0.04	0.293	0.03	252.3	0.05
16S rRNA gene 97% cutoff	1.826	<0.01	0.141	0.02	4.541	0.31
16S rRNA gene 95% cutoff	1.529	0.05	0.079	0.08	6.754	0.39

All three tests are non-parametric multivariate analyses based on dissimilarities among samples. *Permutational multivariate analysis of variance using distance matrices. Significance tests were carried out using *F*-tests based on sequential sums of squares from permutations of the raw data. †Analysis of similarities. Statistic *R* is based on the difference of mean ranks between groups and within groups. The significance of observed *R* is assessed by permuting the grouping vector to obtain the empirical distribution of *R* under the null model ‡Multi-response permutation procedure. Statistic *δ* is the overall weighted mean of within-group means of the pairwise dissimilarities among sampling units. The significance test is the fraction of permuted *δ* that is less than the observed *δ*. § *P* value of corresponding significance test.

shaped by several key plant and soil physical and chemical variables (Supplementary Fig. S3a), including BNPP, C_4 above-ground net primary productivity (ANPP), soil temperature and pH (Supplementary Information). A partial CCA-based variation partitioning analysis indicated that more than 60% of the variations in the community functional composition and structure were explained by plant (32.0%) and soil (25.7%, excluding soil temperature and moisture) variables and their interaction (2.4%; Supplementary Fig. S3b). Soil temperature and moisture alone can directly explain 16.7% of the variation in community functional structure (Supplementary Fig. S3b). Together, these results indicated that the composition, structure and potentially functional activity of microbial communities under experimental warming were significantly different from those of the control, and these differences are strongly controlled by above-ground plant and soil environmental conditions. To the best of our knowledge, this is the first comprehensive study at the whole-community level to clearly demonstrate the changes in the composition and functional structure of microbial communities in response to warming in a grassland ecosystem.

The warming treatment increased soil respiration (autotrophic and heterotrophic respiration) significantly ($P < 0.01$; Fig. 1f). More than half of the soil respiration (59.0% and 57.6% for control and warming, respectively) was from heterotrophic respiration, indicating that microbial activities played substantial roles in soil CO_2 efflux. In agreement with previous reports[17,19], the temperature sensitivity of heterotrophic respiration (Q_{10}) was reduced under warming in this study (Supplementary Fig. S4). The estimated Q_{10} of heterotrophic soil respiration for the warming plots ($Q_{10} = 2.96$) was significantly ($P < 0.0001$) lower than that of control plots ($Q_{10} = 3.22$), indicating that respiratory acclimation has occurred after eight years of warming. The decrease of the Q_{10} value itself in the warming plots would have caused an average of 14.5% (from 13.9 to 15.5%) reduction in heterotrophic respiration, compared with the scenarios without acclimation. These results are also supported by several previous reports[20–22].

Reduced temperature sensitivity of soil respiration seemed to not be due to substrate depletion. The measured labile C in soil samples collected in 2008 was even slightly (7.2%) higher under warming, although not significantly (Fig. 1b). Furthermore, if the substrate is depleted under warming, microbial biomass would be expected to decrease. However, the microbial biomass was significantly higher under warming as measured by phospholipid fatty acid (PLFA) analysis ($P = 0.03$; Fig. 1c). Moreover, the total abundances of detected genes from bacteria and fungi were also marginally higher under warming as measured by GeoChip ($P = 0.08$ for bacteria and 0.06 for fungi, one-tailed paired *t*-test; Fig. 1d). The above results indicated that decreased temperature sensitivity of soil respiration was not due to substrate depletion, but probably because of the changes in microbial community composition and structure. However, further mechanistic studies are needed to establish direct links between community changes and respiratory acclimation.

Second, long-term experimental warming has differential impacts on various microbial functional groups involved in C decomposition. Not all microbial groups/populations were equally stimulated by warming. Most of the populations/genes for labile-C (typically with a turnover time <5 years; ref. 23) degradation were significantly increased under warming, including those for degrading starch, hemicellulose, cellulose and chitin (Fig. 3). Furthermore, many of the genes involved in labile-C degradation such as for exoglucanase, arabinofuranosidase, α-amylase and cyclomaltodextrinase showed significant correlations ($r_M = 0.29$–0.38, $P = 0.003$–0.032) with C_4 but not with C_3 plant biomass based on the Mantel test. Consistently, the physiological activities of the labile-C-degrading genes were greatly stimulated under warming, as revealed by BIOLOG analysis (Supplementary Fig. S5). The labile substrates such as amino acids, carboxylic acids and polymers (glycogen, α-cyclodextrin, Tween 80 and Tween 40) were degraded more quickly ($P < 0.07$) by the samples from warming plots than controls (Supplementary Fig. S5).

However, the populations/genes involved in decomposing relatively recalcitrant C (for example, lignin with 5–12 years turnover time in grassland[24]) were not affected by warming. DNAs from 2007 (Fig. 3) and 2008 (Supplementary Fig. S6) analyses revealed that the genes encoding lignin peroxidase, manganese peroxidase and glyoxyl oxidase remained unchanged under warming. The C/N ratio of C_4 plants (63.0) was significantly higher than that of C_3 plants (35.0; $P < 0.001$) measured in 2008. Thus, although warming led to a higher input of low-quality litter into the soil through the plant community shift towards more C_4 species, it apparently did not stimulate the changes to the corresponding microbial populations. Furthermore, although more C_4 plant tissues containing higher lignin content[25] were incorporated into soils and different soil aggregates and fractions (Supplementary Table S1), none of the lignin-degradation genes showed significant correlations with C_4 plant biomass, and no significant difference in the fungal gene abundance was observed between warming and the control either. In addition, the activities of some key soil enzymes such as phenol oxidase remained unchanged under warming, and the activity of peroxidase was even significantly lower under warming than the control ($P = 0.04$; Fig. 1e).

Such differential impacts on the populations/genes for degrading labile and relatively more recalcitrant C could be important in maintaining the long-term stability and storage of ecosystem C. Consistent with the molecular data, several lines of evidence indicated that warming could have little impact on recalcitrant soil C dynamics in this site. First, there is no observable difference in the recalcitrant-C pool between warming and the control (Fig. 1b). Second, no differences were observed for δ^{13}C (Supplementary Fig. S7a) and δ^{15}N (Supplementary Fig. S7b) values in the mineral

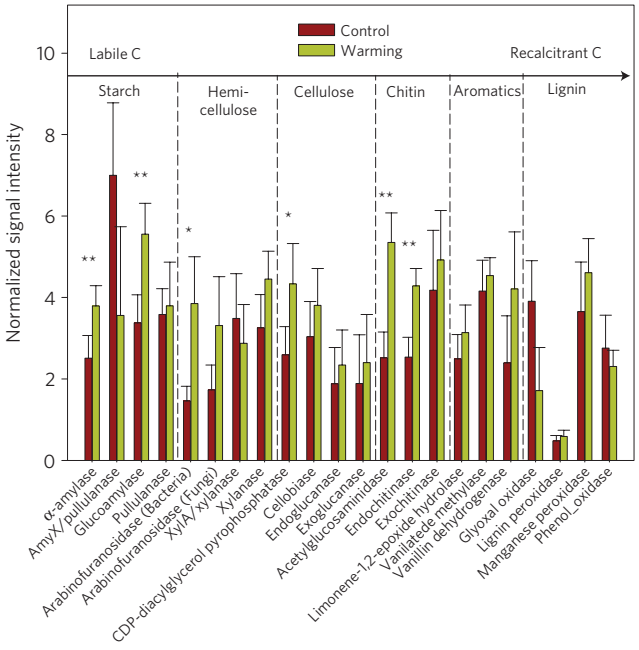

Figure 3 | The normalized average signal intensity of detected C-degradation genes under warming and the control. Signal intensities were the average abundances of detected genes under warming or control plots, normalized by the probe number of each gene. The complexity of C is presented in order from labile to recalcitrant C. Error bars represent standard error ($n = 6$). The differences between warming and the control were tested by two-tailed paired *t*-tests (see Fig. 1 caption).

soil organic matter (SOM) fraction, which accounted for ~80% of the total SOM across all aggregate sizes measured (53–250 μm, 250–2,000 μm and >2,000 μm). As soil C associated with mineral SOM is more recalcitrant, the recalcitrant portion of soil C in this grassland ecosystem seems to be stable under warming. In addition, the δ^{15}N value for the whole soil under warming was marginally higher than those of the control ($P = 0.09$, one-tailed paired *t*-test; Fig. 2c), implying a possible link to increased soil C transformation and humification[26], which stabilizes C by transforming organic matter into humus.

Finally, it seems that warming not only stimulates labile-C-decomposition genes but also enhances genes in nutrient-cycling processes such as denitrification, N fixation, nitrification, N mineralization (Fig. 2a) and phosphorus utilization (Supplementary Fig. S8 and Supplementary Information), which is consistent with the general notion that warming enhances nutrient cycling[6]. The abundance of most key genes involved in N cycling was significantly higher under warming than the control (Fig. 2a). All of the key genes involved in denitrification except *nar*G increased significantly in abundances ($P < 0.05$) under warming (Fig. 2a). Consistent with the gene abundance data, the δ^{15}N values in soil samples from warming plots ($\delta^{15}N = 2.93 \pm 0.7‰$) were marginally higher ($P = 0.09$, one-tailed *t*-test) than that from the control ($\delta^{15}N = 2.7 \pm 1.0‰$; Fig. 2c), implying possible accelerating N-process rates and more N product from microbially mediated processes escaping from the soil system, such as N_2O and N_2 from denitrification. Our laboratory incubation provided direct evidence to support the gene abundance data by showing that denitrification rates for 25 days marginally significantly increased under warming ($P < 0.10$; Fig. 2d). In addition, on the basis of a one-tailed paired *t*-test, the concentrations of NH_4^+ and total available N in the field soils were lower under warming at *P* values of 0.07 and 0.10 (Fig. 2b), respectively, implying a possible N loss through denitrification and/or enhanced plant uptake although the total N remained unchanged. Warming also stimulated the N-fixation gene. The *nif* H gene was much more abundant under warming than the control (Fig. 2a). Interestingly, the magnitude of the gene abundance ratio (warming/control) was about 3–5 times higher for N fixation (*nif* H) than for denitrification (*nor*B, *nos*Z; Fig. 2a). The differential increase of the genes for N fixation and denitrification could lead to higher N availability[6,21]. In fact, *nif* H genes showed a significant positive correlation to soil N availability (ammonium concentration, $r_M = 0.29$, $P = 0.03$) and total soil organic N ($r_M = 0.30$, $P = 0.02$) as well as total soil organic C ($r_M = 0.24$, $P = 0.05$). Thus, increased N fixation could counteract the potential higher N loss due to denitrification and nitrate leaching. As a result, the total N remained unchanged under warming (Fig. 2b). However, although the significant increase in the gene abundance of nutrient-cycling processes under warming may potentially lead to accelerating nutrient-cycling-process rates, more systematic, in-depth studies are needed to determine the rates and extent of various nutrient-cycling processes stimulated, and their impacts on the overall soil nutrient dynamics in this ecosystem.

Our results highlighted at least three possible major mechanisms by which the microbial community mediates carbon-cycle feedbacks to climate warming. The first is through changes in the microbial community composition and structure, which are driven largely by the warming-induced changes in plant community structure and soil physiochemical environments[9]. The shifted microbial communities may have resulted in the reduced temperature sensitivity under warming, which could have decreased C loss by ~10% or about 95 g C m^{-2} yr^{-1}. The second is by differential impacts on different microbial functional groups. Warming increased the abundance of microbial functional populations for labile- but not recalcitrant-C degradation even though more recalcitrant C was input into soils. Such differential effects are critical not only for rapid turnover of plant nutrients (for example, recycling the N bound to labile C) to meet the demand of increased plant growth, but also for long-term ecosystem C sequestration by maintaining the stability of soil C stocks. The last is by enhancing nutrient-cycling processes to promote plant nutrient use efficiency and plant growth. In this experimental site, N mineralization was demonstrated to increase several fold[27] and soil N pools were found to be depleted by 14% (ref. 28) in the warming plots in comparison with that in the control. Thus, stimulation of N-cycling processes, particularly N fixation and N availability through recycling N bound to labile C, would offset N loss under warming as well as maintain the warming stimulation of plant growth. In short, all of these three microbially mediated mechanisms would weaken the positive feedback between the terrestrial C cycle and climate warming. Without such mechanisms, more soil C loss would have occurred in the warming plots. Moreover, the microbially mediated changes in C and nutrient processes as observed in this study have critical implications for projecting future climate warming. Although microorganisms play critical roles in ecosystem functioning and regulating the responses of ecosystems to climate change, they are rarely explicitly considered in carbon–climate models[29]. Our results in this study indicated that whether microbially mediated feedback is positive or negative depends on which microbial groups and associated functions are stimulated and to what magnitude. Thus, to improve the predictions of ecosystem feedbacks to climate warming, it is important to consider various types of feedback mechanism resulting from the changes in microbial composition and structure, at least, at the level of microbial groups with distinct functions.

Methods

This study was conducted in a tall-grass prairie ecosystem in the US Great Plains in Central Oklahoma (34°59′ N, 97°31′ W; refs 9,16). The warming plots (six replicates) have been continually warmed approximately 2 °C since November 1999. Routine plant and soil analyses were carried out as previously described[9]. Soil respiration was measured once or twice a month between 10:00 and 15:00

(local time) using a LI-COR 6400 portable photosynthesis system attached to a soil CO_2 flux chamber (LI-COR). The Q_{10} values for monthly heterotrophic soil respiration in control and warming plots were estimated by an inverse analysis method. To determine whether long-term warming affects microbial community structure, several metagenomic and conventional microbial analyses were carried out, including PLFA analysis for 2008 samples, which provides information on the physiological activity of microbial communities; enzyme activity for 2008 samples; BIOLOG analysis to examine substrate utilization profile patterns; labile C and total soil organic C analyses[9] for 2008 samples; functional gene array (that is, GeoChip 3.0) analysis for 2007 samples, which measures the functional structure of microbial communities; and 16S ribosomal RNA gene-based targeted pyrosequencing for 2007 samples, which assesses the phylogenetic composition of microbial communities. Community DNA (5 g soil collected from 0 to 20 cm depth in April 2007) was extracted and purified with freeze-grinding mechanical lysis[30]. Pyrosequencing of PCR-amplified V4–V5 hypervariable regions of the 16S rRNA was carried out with the 454 FLX Systems with a sample-tagging approach. The raw 454 sequences were classified into different taxonomical levels and operational taxonomic units using the Ribosomal Database Project pyrosequencing pipeline[15]. GeoChip analyses were carried out by Microarray Data Manager on our website (http://ieg.ou.edu/microarray/). The microbial diversity indices were analysed by R software version 2.9.1 (the R foundation for statistical computing). To test the significance of the differences between warming and control treatment for various variables, two-tailed paired *t*-tests were employed by Microsoft Excel 2010. One-tailed paired tests were also carried out to improve the power of the *t*-test for certain ecosystem parameters that are expected to increase or decrease under warming on the basis of our previous knowledge. For multivariate gene data, DCA, CCA and three non-parametric analyses (analysis of similarity (ANOSIM), non-parametric multivariate analysis of variance (adonis) using distance matrices, and a multi-response permutation procedure (MRPP)) were carried out by R software version 2.9.1 as well. Details for all methods are provided in the Supplementary Information.

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Author contributions

All authors contributed intellectual input and assistance to this study and manuscript preparation. The original concept and experimental strategy were developed by J.Z. and L.W. Sampling collections, DNA preparation, GeoChip and pyrosequencing analysis were carried out by Y.D., J.X. and L.W. K.X. carried out soil chemical analysis and various statistical analyses with Y.D., and S.F. carried out modelling analysis. S.D. carried out soil enzyme analysis. Z.H., Y.D. and J.D.V.N. assisted with GeoChip and sequencing analysis. J.Z. and Y.L. guided all data analysis and integration. J.Z. and K.X. wrote the paper with help from Y.L. and Z.H.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/natureclimatechange. Reprints and permissions information is available online at http://www.nature.com/reprints. Correspondence and requests for materials should be addressed to J.Z.