nature climate change

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41558-018-0254-2

In the format provided by the authors and unedited.

Climate warming leads to divergent succession of grassland microbial communities

Xue Guo[1,2,3,4,11], Jiajie Feng[2,3,11], Zhou Shi[2,3,11], Xishu Zhou[1,2], Mengting Yuan[2,3,5], Xuanyu Tao[2,3], Lauren Hale[2,3], Tong Yuan[2,3], Jianjun Wang[2,3], Yujia Qin[2,3], Aifen Zhou[2,3], Ying Fu[2,3], Liyou Wu[2,3], Zhili He[2,3], Joy D. Van Nostrand[2,3], Daliang Ning[2,3,4], Xueduan Liu[1], Yiqi Luo[3,6,7], James M. Tiedje[8], Yunfeng Yang[2,4]* and Jizhong Zhou[2,3,4,9,10]*

[1]School of Minerals Processing and Bioengineering, Central South University, Changsha, China. [2]Institute for Environmental Genomics, University of Oklahoma, Norman, OK, USA. [3]Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, USA. [4]State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing, China. [5]Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA, USA. [6]Center for Ecosystem Science and Society, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, USA. [7]Department of Earth System Science, Tsinghua University, Beijing, China. [8]Center for Microbial Ecology, Michigan State University, East Lansing, MI, USA. [9]School of Civil Engineering and Environmental Sciences, University of Oklahoma, Norman, OK, USA. [10]Earth and Environmental Sciences, Lawrence Berkeley National Laboratory, Berkeley, CA, USA. [11]These authors contributed equally: Xue Guo, Jiajie Feng, Zhou Shi.
*e-mail: yangyf@tsinghua.edu.cn; jzhou@ou.edu

Climate Warming Leads to Divergent Succession of Grassland Microbial Communities

Supplementary Figures and Tables

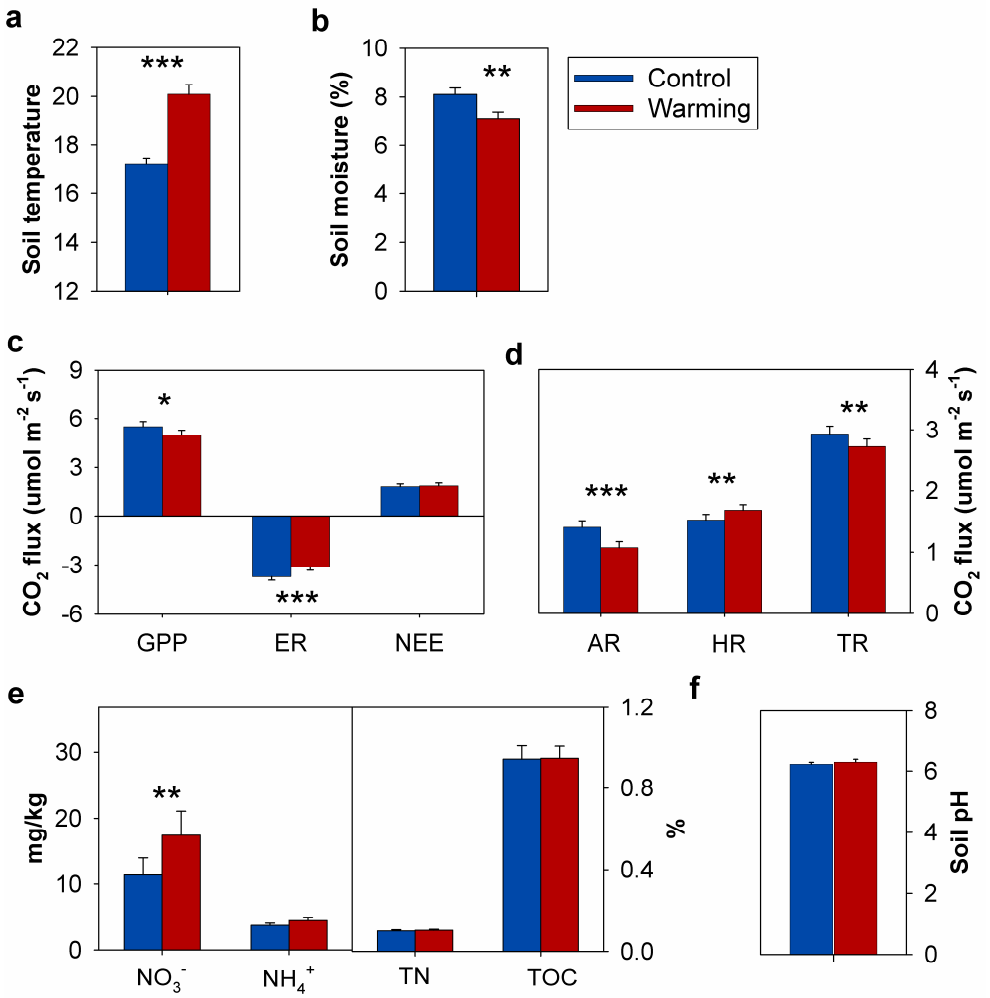


Fig. S1. Warming effects on a series of plant and soil variables across 6 years. (**a**) Effects of warming on soil temperature in the depth of 7.5 cm; (**b**) Soil moisture in the surface layer (0-15 cm); (**c**) Ecosystem Carbon (C) fluxes and soil respirations, which were estimated on the basis of the C amount from CO_2 emissions: gross primary productivity (GPP), ecosystem respiration (ER), and net ecosystem C exchange (NEE). Positive values indicate C sink, and negative values represent C source. (**d**) Soil respirations *in situ* including autotrophic respiration (AR), heterotrophic respiration (HR), total soil respiration (TR); **(e)** Soil nitrate (NO_3^-), ammonia (NH_4^+), total N (TN) and total organic C (TOC); and **(f)** soil pH. The differences between warming and the control were tested by repeated measures ANOVA, indicated by *** when $p < 0.01$, ** when $p < 0.05$, * when $p < 0.10$.

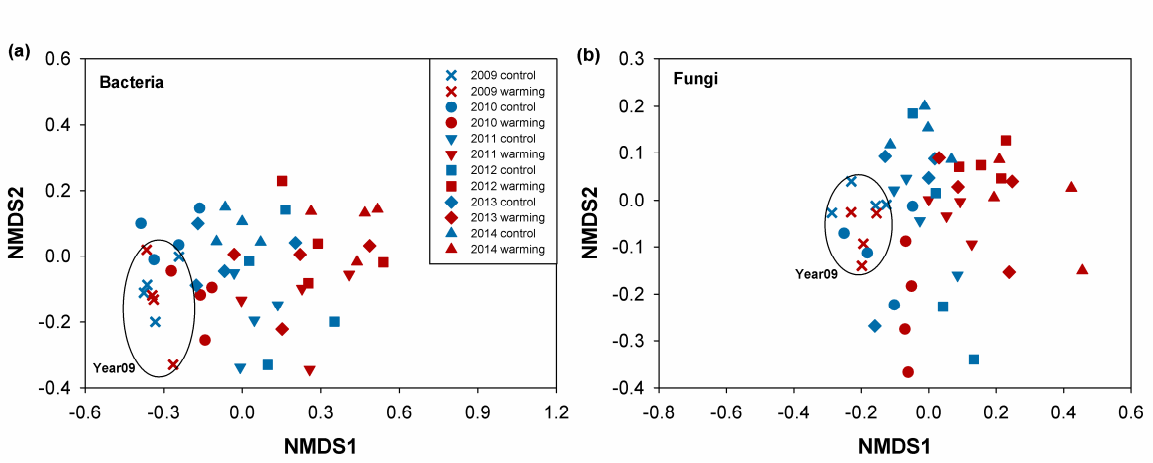


Fig. S2. Non-metric multidimensional scaling (NMDS) ordination of the temporal changes in microbial communities under warming and control treatments. The analysis was performed based on Bray-Curtis dissimilarity. For bacteria (**a**) and fungi (**b**), warmed and control samples were clustered together in the first year (2009). The warmed samples generally tended to cluster together and were separated from control samples by NMDS1 in the following five years (2010-2014).

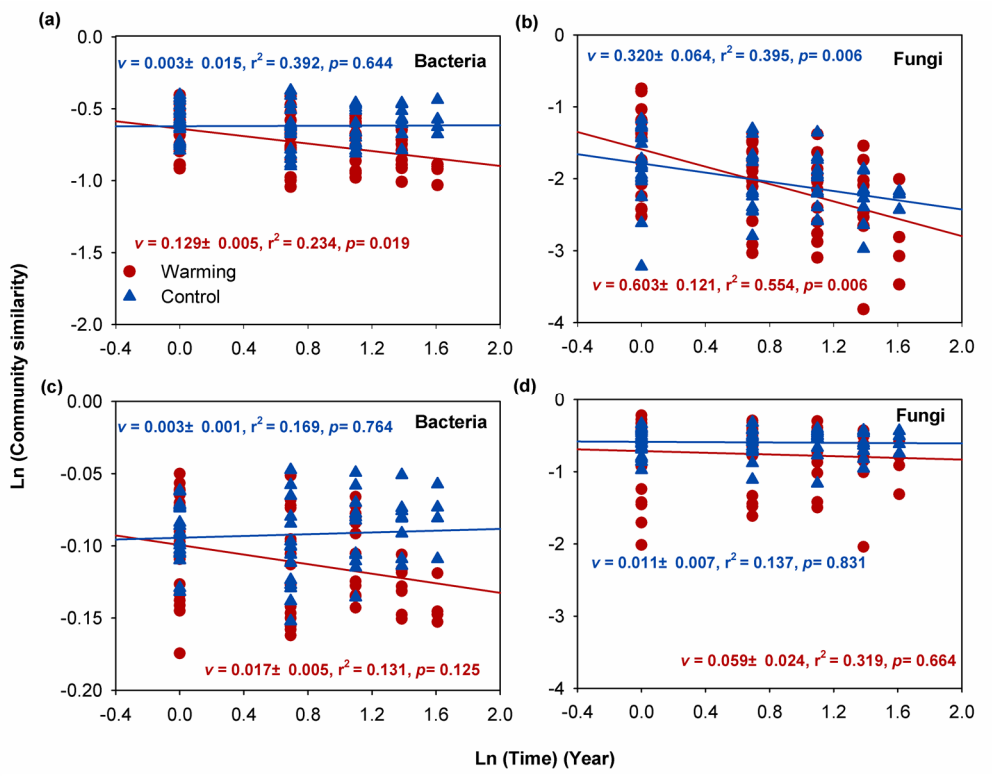


Fig. S3. The time-decay relationships (TDRs) of bacteria (a, c) and fungi (b, d) under warming and control. (a, b) Bray-Curtis; **(c, d)** Weighted UniFrac dissimilarity metrics. Details are described in the legend of Fig. 1.

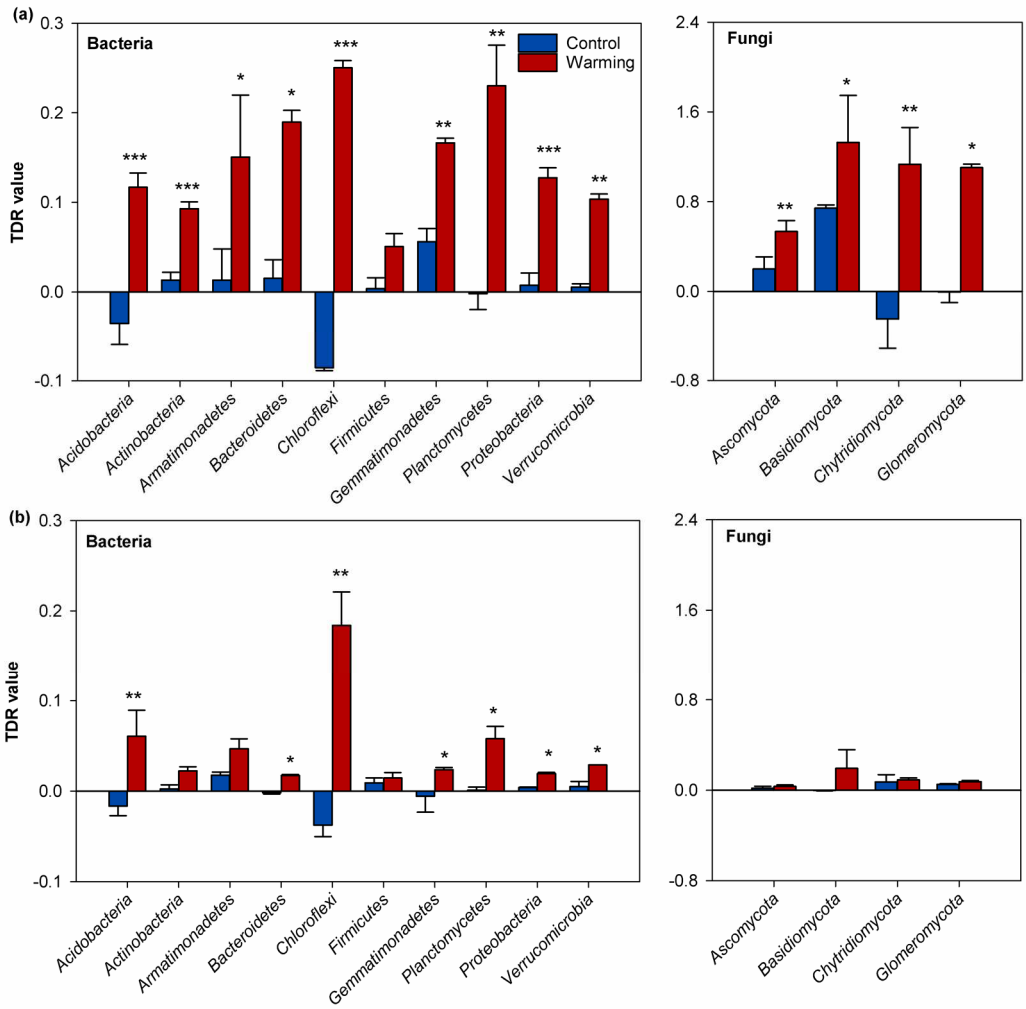


Fig. S4. TDR values of microbial communities among different phylogenetic groups under warming and control based on Bray-Curtis (a) and weighted UniFrac (b) metrics. TDR values in different phyla were calculated from linear mixed model as described in Fig. 1. The bars represent standard errors. The significance of TDR values under different treatments are based on permutation test and indicated by *** when $p < 0.01$, ** when $p < 0.05$, * when $p < 0.10$.

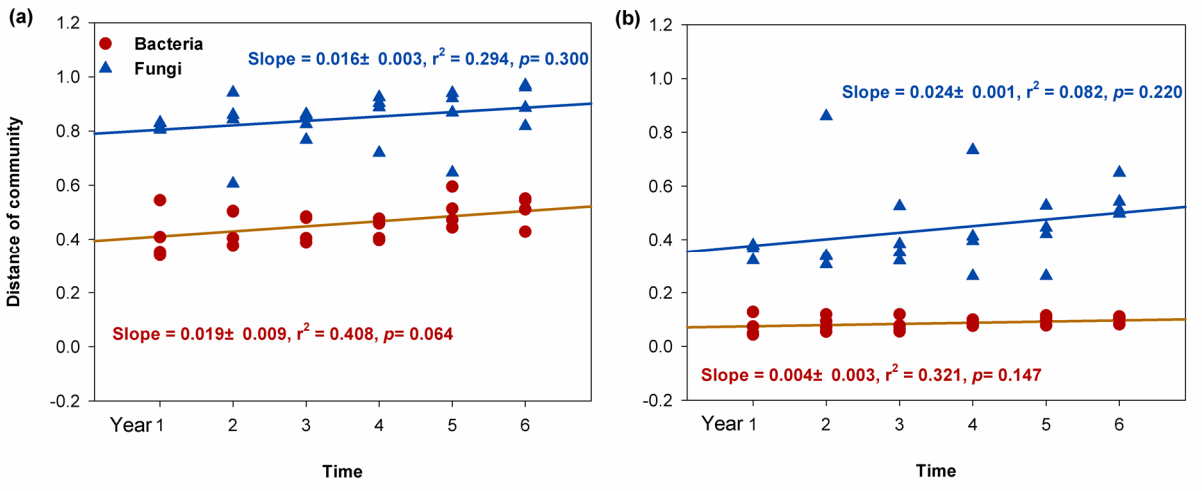


Fig. S5. Overall community differences between warming and control. (**a**) Bray-Curtis dissimilarity; (**b**) Weighted UniFrac dissimilarity metrics. Community distances of bacteria and fungi between warming and control increased linearly with time. The first year is 2009. The slopes were calculated from linear mixed model (LMM) as in Fig. 3. The different color lines show fixed effects in LMM.

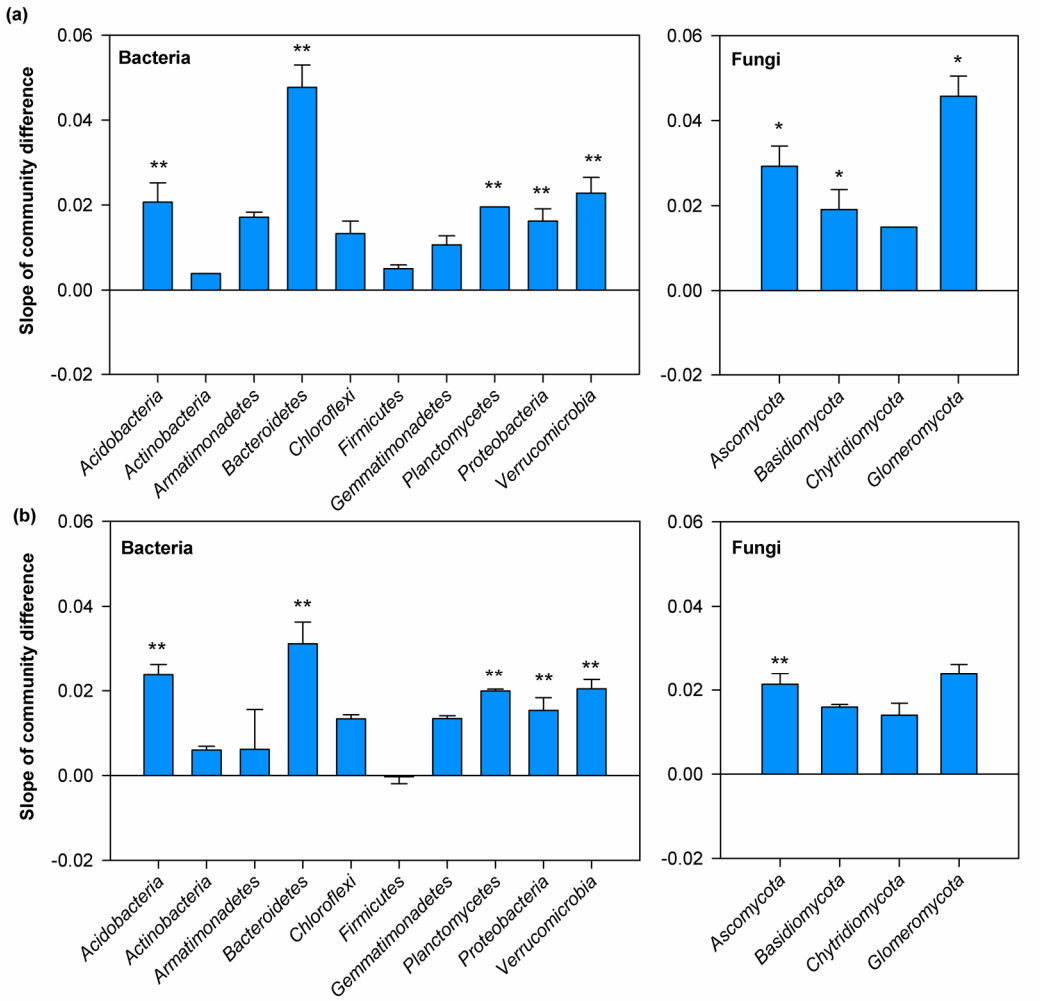


Fig. S6. The slopes of community differences between warming and control treatments among different phylogenetic lineages. (a) Sorensen; **(b)** Unweighted UniFrac dissimilarity metrics. The temporal slope of community difference in different phyla were calculated from linear mixed model (LMM) as in Fig. 3. The significance of each LMM is based on permutation test and indicated by ** when $p < 0.05$, * when $p < 0.10$. The information for other diversity metrics (Bray-Curtis and Weighted UniFrac) is shown in Supplementary Fig. S7.

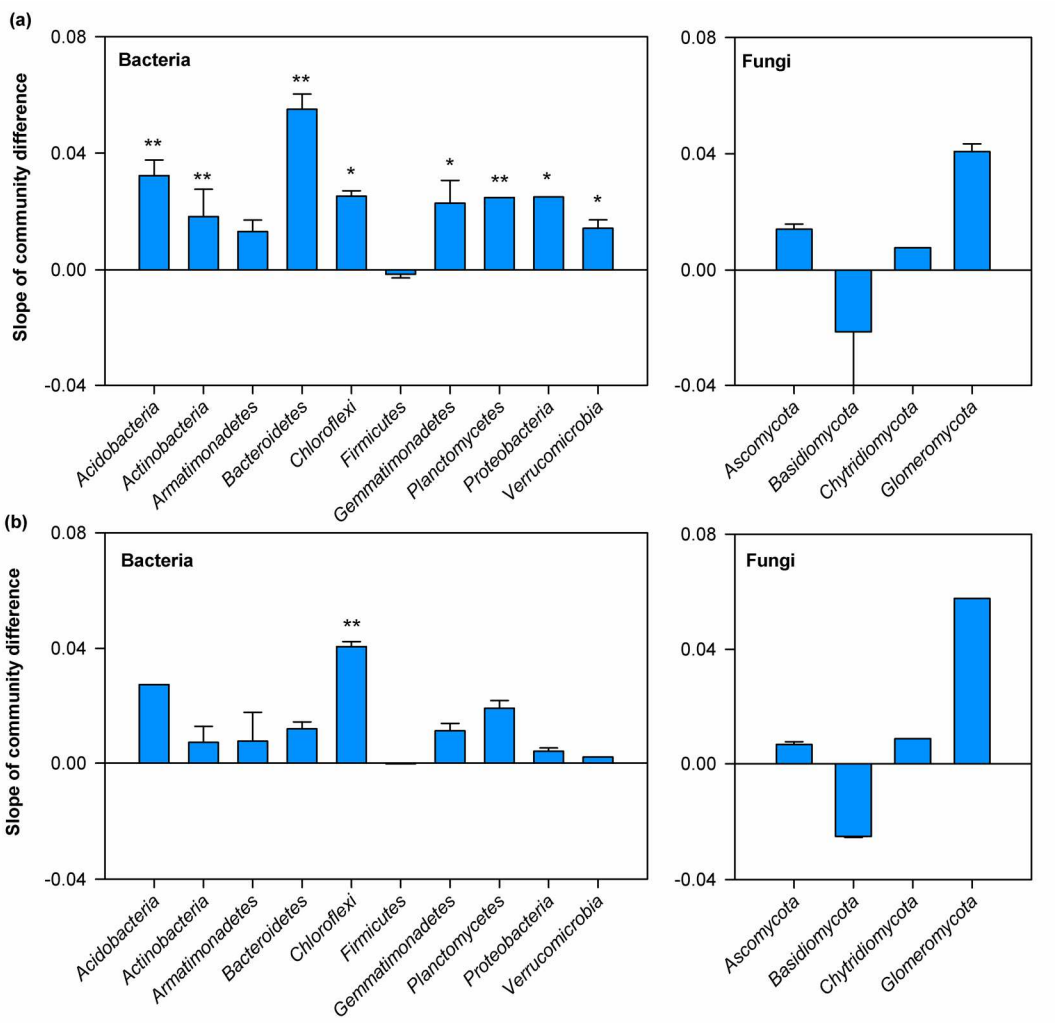


Fig. S7. The slope of community difference between warming and control among different phylogenetic lineages. (a) Bray-Curtis; **(b)** Weighted UniFrac dissimilarity metrics. The slope of community difference in different phyla were calculated from linear mixed model (LMM) as in Fig. 3. The significance of each LMM is based on permutation test and indicated by * when $p < 0.05$, * when $p < 0.10$.

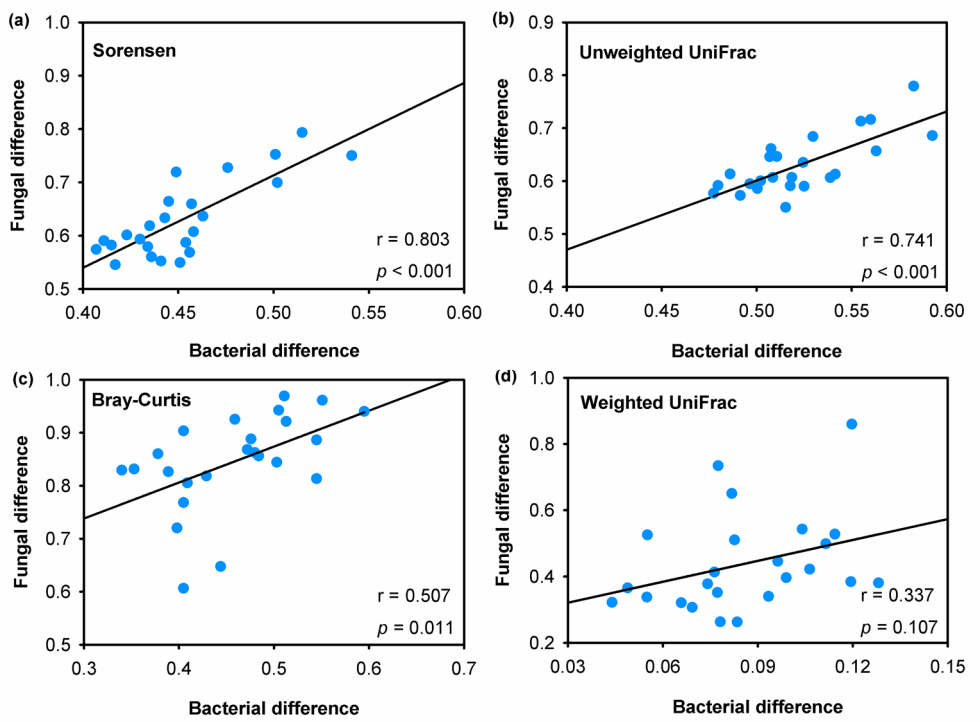
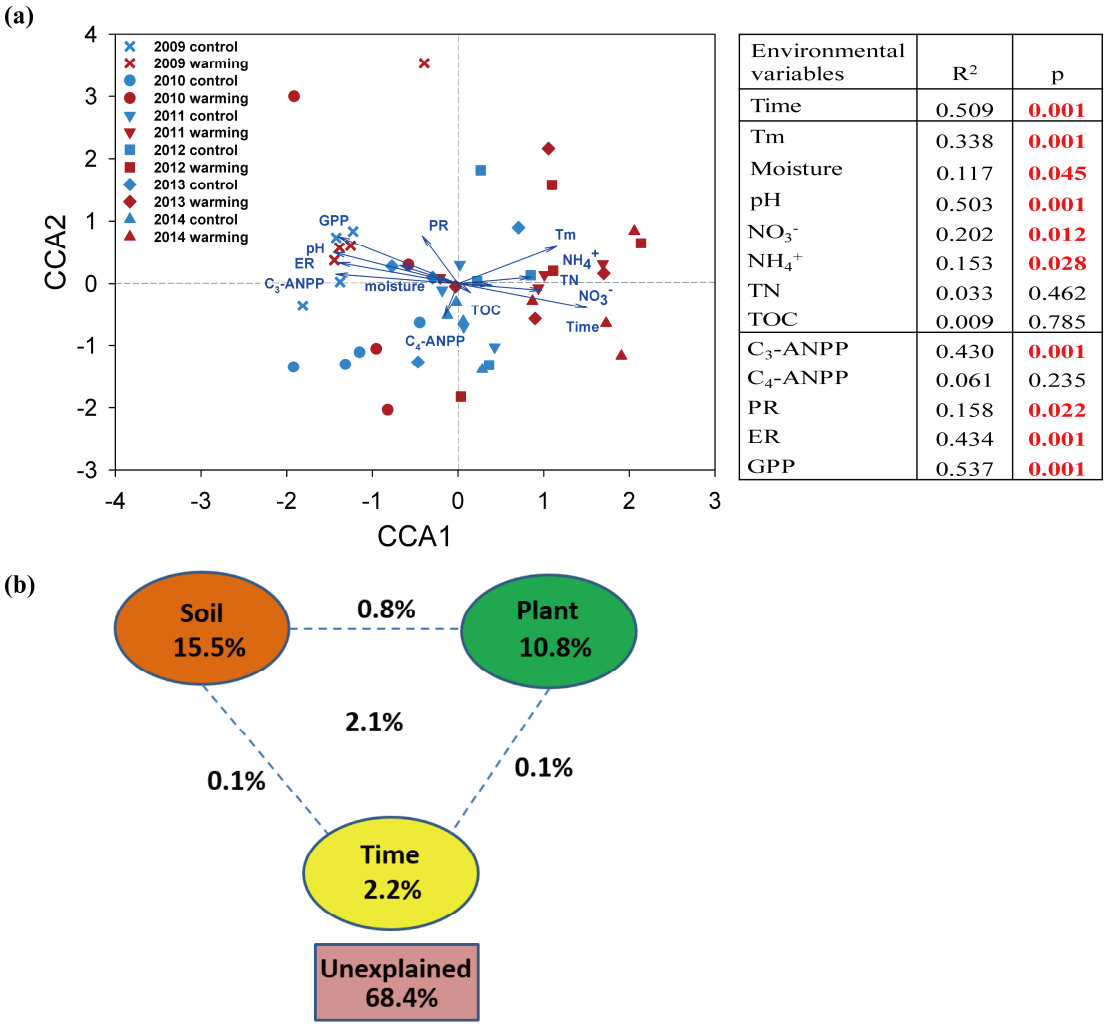
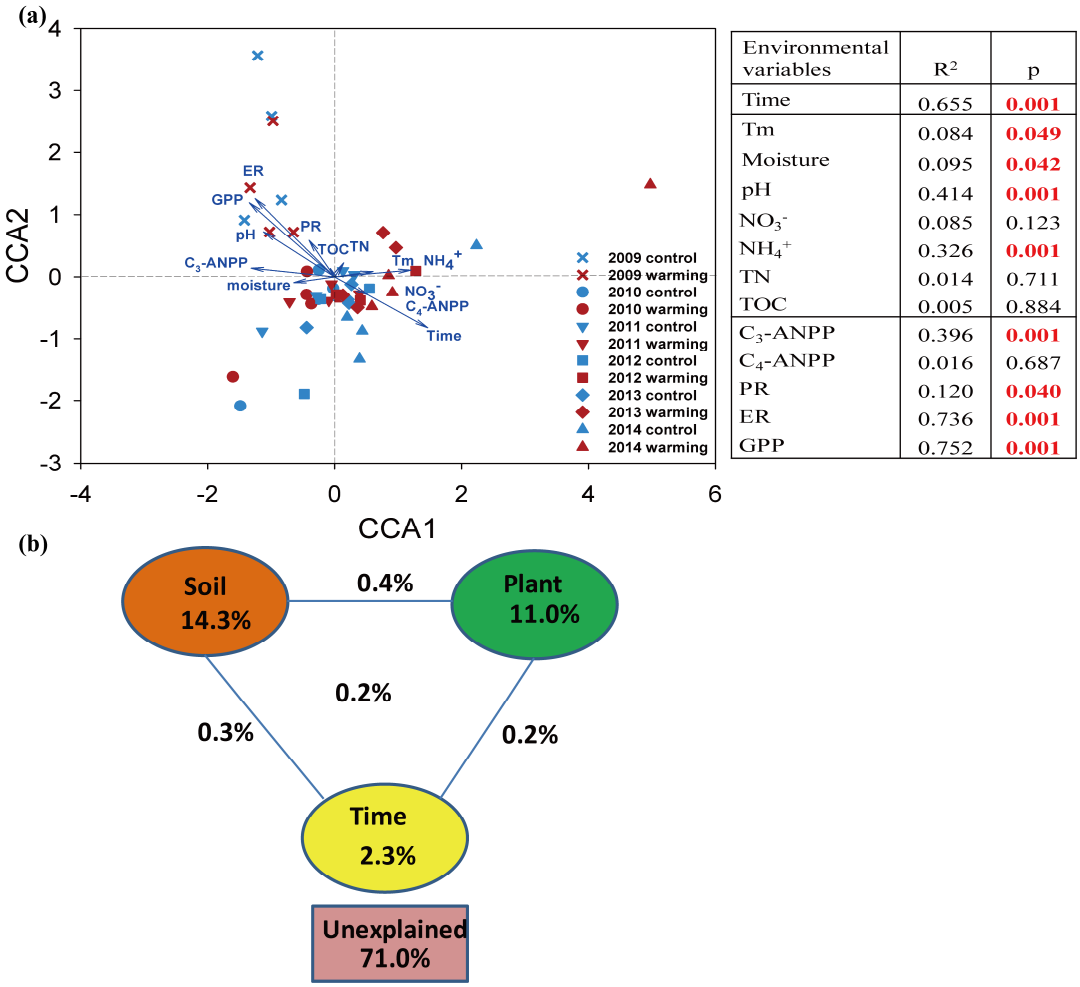


Fig. S8. Relationships between bacterial and fungal community differences in warmed and control plots. (**a, c**) taxonomic diversity; (**b, d**) phylogenetic diversity.



Environmental variables	R^2	p
Time	0.509	**0.001**
Tm	0.338	**0.001**
Moisture	0.117	**0.045**
pH	0.503	**0.001**
NO_3^-	0.202	**0.012**
NH_4^+	0.153	**0.028**
TN	0.033	0.462
TOC	0.009	0.785
C_3-ANPP	0.430	**0.001**
C_4-ANPP	0.061	0.235
PR	0.158	**0.022**
ER	0.434	**0.001**
GPP	0.537	**0.001**

Fig. S9. Constrained ordination analysis of bacterial communities. (a) Canonical correspondence analyses (CCA) of 16S rRNA gene sequence data and environmental attributes. Bacterial community composition and structure were significantly shaped by plant related factors: C_3 and C_4 aboveground net plant productivities (ANPP), plant richness (PR), gross primary productivity (GPP), ecosystem respiration (ER), by soil related factors: soil nitrate (NO_3^-) and ammonia (NH_4^+) contents, total organic carbon (TOC), total nitrogen (TN), soil pH, soil temperature (Tm) and moisture (MS), and by time. The insert table showed the significances of each environmental variable in explaining the variations of bacteria community. **(b)** CCA-based variation partitioning analysis (VPA) showed the relative proportions of bacterial community variations that can be explained by different types of environmental factors. The numbers within the circles showed the variation explained by each group of environmental factors alone. The numbers between the circles showed the interactions of the two factors on either side and number in the center of the interactions of all three factors.



Environmental variables	R^2	p
Time	0.655	**0.001**
Tm	0.084	**0.049**
Moisture	0.095	**0.042**
pH	0.414	**0.001**
NO_3^-	0.085	0.123
NH_4^+	0.326	**0.001**
TN	0.014	0.711
TOC	0.005	0.884
C_3-ANPP	0.396	**0.001**
C_4-ANPP	0.016	0.687
PR	0.120	**0.040**
ER	0.736	**0.001**
GPP	0.752	**0.001**

Fig. S10. Constrained ordination analysis of fungal communities. (a) Canonical correspondence analyses (CCA) of ITS sequence data and environmental attributes. **(b)** CCA-based variation partitioning analysis (VPA) showed the relative proportions of fungal community variations that can be explained by different types of environmental factors. Details are described in Supplementary Fig. S9.

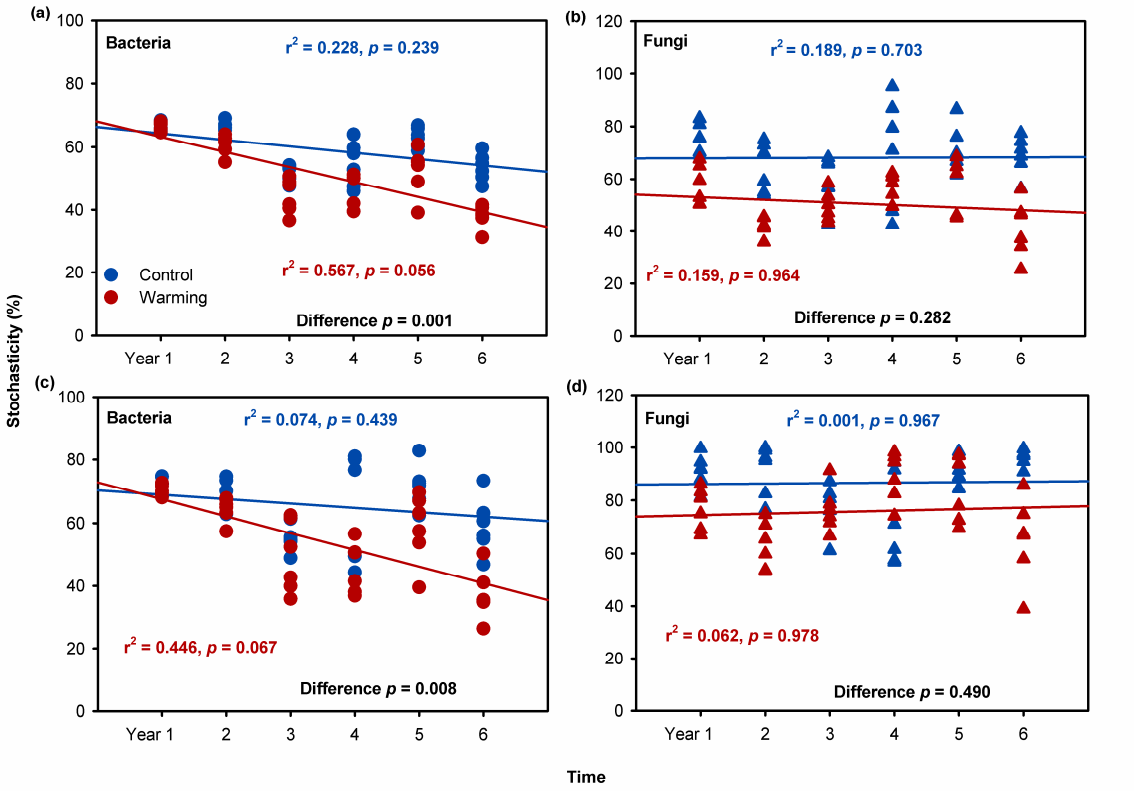


Fig. S11. Temporal changes of stochasticity under warming (red) and control (blue) based on taxonomic (a, b) and phylogenetic diversity (c, d). Warming substantially decreased the relative importance of stochastic processes for bacteria but not for fungi although the trend existed. The pairwise stochasticity index within each treatment was fitted to linear mixed models (LMM) with a fixed effect of time and a random intercept and slope effect among different pairs of plots within the same treatment. The r^2 values were calculated (details in method), reflecting variance explained by the whole model. Significance test of each LMM and the slope difference between warming and control are based on permutation test. The different lines in each plot showed the fixed effect of LMM. The first year is 2009.

Table S1. Summary of permutational multivariate analysis of warming, year, block on microbial communities.

Datasets	Adonis			
	Df	*F*	R^2	*p*
Bacteria (16S)				
Warming	1	2.611	0.044	**0.004**
Year	5	2.778	0.236	**0.001**
Block	3	0.163	0.083	**0.002**
Year*Block	15	0.956	0.244	0.664
Residuals	23	-	1.000	-
Fungi (ITS)				
Warming	1	2.005	0.039	**0.001**
Year	5	1.608	0.156	**0.001**
Block	3	1.375	0.080	**0.004**
Year*Block	15	0.960	0.279	0.773
Residuals	23	-	1.000	-

For the permutational multivariate analysis of variance (Adonis), the one-way repeated-measures ANOVA model was set as "dissimilarity~ warming+ block×year" by using function adonis in R package vegan. Significant *p* values (< 0.05) are bolded.

Table S2. TDR values (v) of different phylogenetic groups based on taxonomic diversity and their significant differences between warming and control determined by permutation tests.

	Sorensen					Bray-Curtis				
	Control (C)		Warming (W)		C vs. W[2]	Control (C)		Warming (W)		C vs. W[2]
	v[1]	r^2	v	r^2		v	r^2	v	r^2	
Bacteria										
Acidobacteria	-0.011±0.014	0.511	0.072±0.011**	0.502	+***[3]	-0.035±0.025	0.518	0.117±0.016**	0.405	+***
Actinobacteria	0.008±0.000	0.121	0.033±0.002**	0.293	+**	0.013±0.009	0.278	0.093±0.008**	0.476	+***
Armatimonadetes	0.004±0.004	0.199	0.130±0.029*	0.221	+*	0.013±0.035	0.225	0.151±0.069*	0.263	+*
Bacteroidetes	0.046±0.016	0.156	0.164±0.010*	0.273	+*	0.015±0.021	0.064	0.190±0.013*	0.257	+*
Chloroflexi	-0.011±0.004	0.437	0.212±0.016**	0.381	+***	-0.085±0.004	0.401	0.250±0.008**	0.426	+***
Firmicutes	0.008±0.003	0.454	0.073±0.022**	0.251	+**	0.004±0.012	0.407	0.051±0.015	0.297	+
Gemmatimonadetes	0.047±0.009	0.270	0.104±0.015**	0.245	+*	0.056±0.015	0.353	0.166±0.005**	0.424	+**
Planctomycetes	-0.012±0.013	0.353	0.161±0.000**	0.267	+***	-0.002±0.018	0.319	0.230±0.046*	0.268	+**
Proteobacteria	0.032±0.002	0.238	0.099±0.007**	0.383	+**	0.008±0.014	0.082	0.128±0.012**	0.403	+***
Verrucomicrobia	-0.008±0.004	0.224	0.092±0.019**	0.416	+**	0.005±0.004	0.322	0.104±0.006**	0.399	+**
Fungi										
Ascomycota	0.092±0.011**	0.516	0.232±0.033**	0.817	+**	0.201±0.108*	0.308	0.534±0.098**	0.382	+**
Basidiomycota	0.166±0.017**	0.341	0.367±0.025**	0.379	+*	0.743±0.030*	0.319	1.328±0.419**	0.438	+*
Chytridiomycota	-0.209±0.225	0.096	0.857±0.005	0.142	+**	-0.246±0.268	0.076	1.134±0.329	0.142	+**
Glomeromycota	0.058±0.056	0.155	0.751±0.101	0.380	+*	-0.005±0.093	0.135	1.104±0.033	0.391	+*

[1] The TDR values (v), r^2 values, and significance were calculated based on linear mixed model as described in Fig. 1 and Fig. 2.

[2] The observed v difference between warming and control was compared with the v difference in permuted datasets to obtain the p value. + indicates v increased under warming, - indicates v decreased under warming.

[3] *** when $p< 0.01$, ** when $p< 0.05$, * when $p< 0.10$.

Table S3. TDR values of different phylogenetic groups based on phylogenetic diversity and their significant difference between warming and control treatments determined by permutation tests.

	Unweighted UniFrac					Weighted UniFrac				
	Control (C)		Warming (W)		C vs. W[2]	Control (C)		Warming (W)		C vs. W[2]
	v[1]	r^2	*v*	r^2		*v*	r^2	*v*	r^2	
Bacteria										
Acidobacteria	-0.007±0.007	0.362	0.076±0.011**	0.404	+***[3]	-0.017±0.011	0.378	0.061±0.029	0.227	+**
Actinobacteria	0.003±0.001	0.076	0.046±0.006**	0.310	+**	0.002±0.005	0.043	0.022±0.005	0.122	+
Armatimonadetes	-0.009±0.025	0.164	0.060±0.005	0.202	+*	0.018±0.004	0.178	0.047±0.011	0.214	+
Bacteroidetes	0.062±0.001	0.184	0.148±0.018*	0.232	+	-0.002±0.002	0.237	0.017±0.002*	0.617	+*
Chloroflexi	-0.008±0.007	0.414	0.146±0.033**	0.401	+***	-0.038±0.013	0.260	0.184±0.037**	0.469	+**
Firmicutes	0.012±0.017	0.125	0.037±0.000	0.047	+	0.009±0.006	0.341	0.014±0.006	0.063	+
Gemmatimonadetes	0.011±0.012	0.145	0.065±0.012**	0.163	+*	-0.006±0.018	0.322	0.024±0.003	0.291	+*
Planctomycetes	-0.007±0.011	0.386	0.110±0.000**	0.307	+***	0.001±0.004	0.286	0.058±0.014	0.218	+*
Proteobacteria	0.019±0.011	0.162	0.094±0.016**	0.439	+***	0.004±0.001	0.035	0.019±0.001*	0.423	+*
Verrucomicrobia	-0.014±0.000	0.249	0.092±0.014*	0.299	+**	0.005±0.006	0.078	0.029±0.000	0.182	+*
Fungi										
Ascomycota	0.062±0.014**	0.433	0.137±0.030***	0.747	+**	0.018±0.017	0.322	0.034±0.012	0.163	+
Basidiomycota	0.093±0.001**	0.283	0.144±0.006***	0.465	+	-0.002±0.003	0.127	0.194±0.164	0.226	+
Chytridiomycota	0.042±0.100	0.313	0.134±0.047	0.091	+	0.072±0.067	0.476	0.092±0.017	0.069	+
Glomeromycota	0.009±0.019	0.163	0.329±0.265	0.654	+**	0.050±0.007	0.163	0.075±0.012	0.559	+

[1] The TDR value (*v*), r^2 and significance were calculated based on linear mixed model as described in Fig. 1 and Fig. 2.

[2] The observed *v* difference between warming and control was compared with the *v* difference in randomized datasets to obtain the *p* value. + indicates *v* increased under warming, - indicates *v* decreased under warming.

[3] *** when $p < 0.01$, ** when $p < 0.05$, * when $p < 0.10$.

Table S4. Temporal change of the differences between warming and control treatments based on taxonomic and phylogenetic diversity of each phylogenetic group.

	Sorensen			Bray-Curtis		
	Slope[1]	r^2	*p*[2]	*Slope*	r^2	*p*
Bacteria						
Acidobacteria	0.021±0.005	0.595	**0.019**	0.032±0.005	0.478	**0.028**
Actinobacteria	0.004±0.001	0.075	0.467	0.018±0.009	0.640	**0.014**
Armatimonadetes	0.017±0.001	0.411	0.153	0.013±0.004	0.177	0.293
Bacteroidetes	0.048±0.005	0.674	**0.021**	0.055±0.005	0.637	**0.014**
Chloroflexi	0.013±0.003	0.301	0.373	0.025±0.002	0.501	**0.097**
Firmicutes	0.005±0.001	0.238	0.467	-0.002±0.001	0.019	0.886
Gemmatimonadetes	0.011±0.002	0.127	0.467	0.023±0.008	0.438	**0.065**
Planctomycetes	0.020±0.001	0.458	**0.019**	0.025±0.001	0.466	**0.023**
Proteobacteria	0.016±0.003	0.706	**0.022**	0.025±0.001	0.339	**0.056**
Verrucomicrobia	0.023±0.004	0.548	**0.019**	0.014±0.003	0.274	**0.091**
Fungi						
Ascomycota	0.029±0.005	0.342	**0.056**	0.014±0.002	0.014	0.458
Basidiomycota	0.019±0.005	0.175	**0.095**	-0.021±0.027	0.001	0.132
Chytridiomycota	0.015±0.001	0.039	0.631	0.008±0.001	0.035	0.794
Glomeromycota	0.046±0.005	0.188	**0.095**	0.041±0.003	0.174	0.125

[1] The temporal slopes and r^2 values were calculated based on linear mixed model as described in Fig. 3.

[2] Significant *p* values (< 0.10) are bolded.

Table S5. Temporal change of the differences between warming and control treatments based on taxonomic and phylogenetic diversity in each phylogenetic group.

	Unweighted UniFrac			Weighted UniFrac		
	Slope[1]	r^2	*p*[2]	*Slope*	r^2	*p*
Bacteria						
Acidobacteria	0.024±0.003	0.565	**0.014**	0.027±0.001	0.429	0.111
Actinobacteria	0.006±0.001	0.162	0.389	0.007±0.006	0.311	0.255
Armatimonadetes	0.006±0.009	0.445	0.106	0.008±0.010	0.383	0.178
Bacteroidetes	0.031±0.005	0.625	**0.028**	0.012±0.002	0.316	0.111
Chloroflexi	0.013±0.001	0.341	0.238	0.041±0.002	0.531	**0.028**
Firmicutes	0.000±0.002	0.084	0.839	0.000±0.001	0.001	0.989
Gemmatimonadetes	0.013±0.001	0.126	0.389	0.011±0.003	0.138	0.545
Planctomycetes	0.020±0.001	0.586	**0.028**	0.019±0.003	0.415	0.132
Proteobacteria	0.015±0.003	0.752	**0.014**	0.004±0.001	0.242	0.389
Verrucomicrobia	0.020±0.002	0.608	**0.028**	0.002±0.001	0.023	0.676
Fungi						
Ascomycota	0.021±0.003	0.358	**0.042**	0.007±0.001	0.004	0.708
Basidiomycota	0.016±0.001	0.253	0.125	-0.025±0.001	0.001	0.708
Chytridiomycota	0.014±0.003	0.037	0.547	0.009±0.001	0.013	0.817
Glomeromycota	0.024±0.002	0.118	0.213	0.058±0.001	0.193	0.208

[1] The temporal slopes and r^2 values were calculated based on linear mixed model as described in Fig. 3.

[2] Significant *p* values (< 0.10) are bolded.

Table S6. Partial Mantel test results to discern correlation between soil microbial community variations and either soil temperature (Soil T) or soil moisture (soil M).

Control for:	Soil T		Soil M		Soil T > Soil M[1]
	Soil M		Soil T		
	r_m	p[2]	r_m	p	
Bacteria	0.166	**0.020**	0.146	**0.045**	√
Acidobacteria	0.215	**0.016**	0.297	**0.002**	×
Actinobacteria	0.122	**0.050**	0.288	**0.003**	×
Armatimonadetes	0.301	**0.001**	0.066	0.198	√
Bacteroidetes	0.219	**0.002**	0.207	**0.006**	√
Chloroflexi	0.228	**0.001**	0.092	0.106	√
Firmicutes	-0.025	0.541	0.001	0.492	×
Gemmatimonadetes	0.162	**0.014**	0.134	**0.030**	√
Planctomycetes	0.312	**0.001**	0.162	**0.005**	√
Proteobacteria	0.195	**0.018**	0.136	0.076	√
Verrucomicrobia	0.145	**0.03**	0.200	**0.009**	×
Fungi	0.045	0.225	0.147	**0.027**	×
Ascomycota	0.088	0.099	0.054	0.233	√
Basidiomycota	0.085	**0.028**	0.098	**0.021**	×
Chytridiomycota	0.044	0.222	0.123	**0.045**	×
Glomeromycota	-0.010	0.546	0.135	**0.036**	×

[1] Comparison of the correlations of soil microbial community variations with for soil temperature or for soil moisture. √ when the r_m for soil temperature was larger than the r_m for soil moisture; × when the r_m soil temperature was smaller than the r_m for soil moisture.

[2] Significant p values (< 0.05) are bolded.

Table S7. Mantel test between the structure of different phylogenetic groups and key ecosystem functional attributes.

Attributes[1]	HR	TR	AR	ER	GPP	NEE
Bacteria	**	**			***	***
Acidobacteria			***		**	
Actinobacteria		**	**	**	**	**
Armatimonadetes		**	**			
Bacteroidetes		**	**			
Chloroflexi						
Firmicutes			**			
Gemmatimonadetes		**	**	**	**	
Planctomycetes	**			***	***	**
Proteobacteria						**
Verrucomicrobia	**		***	***	***	**
Fungi	**	***		***	***	
Ascomycota	**			***	***	
Basidiomycota	**	**	**		**	
Chytridiomycota		**	**	***	***	
Glomeromycota						

[1] Ecosystem functional attributes included heterotrophic respiration (HR), soil total respiration (TR), autotrophic respiration (AR), ecosystem respiration (ER), gross primary productivity (GPP), net ecosystem C exchange (NEE). Significant p values were represented by *** when $p<0.01$ and ** when $p<0.05$.

Table S8. CCA between the structure of each phylogenetic group and each key ecosystem functional attributes.

Attributes[1]	HR	TR	AR	ER	GPP	NEE
Bacteria		**		***	***	**
Acidobacteria		**	***	***	***	***
Actinobacteria		**	**	***	***	**
Armatimonadetes				***	***	
Bacteroidetes		**		**	***	
Chloroflexi		***	**	***	***	
Firmicutes		***	**	***	***	**
Gemmatimonadetes				**	**	
Planctomycetes				***	***	**
Proteobacteria	**			***	***	***
Verrucomicrobia	***		**	**	**	
Fungi	**	***	**	***	***	
Ascomycota	**			***	***	
Basidiomycota				***	***	***
Chytridiomycota				**	**	
Glomeromycota	**	***	**	***	***	***

[1] Ecosystem attributes include soil temperature (Soil T), soil moisture (Soil M), heterotrophic respiration (HR), soil total respiration (TR), autotrophic respiration (AR), ecosystem respiration (ER), gross primary productivity (GPP), net ecosystem C exchange (NEE). Significant p values were represented by *** when $p<0.01$ and ** when $p<0.05$.