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Reduction of microbial diversity in grassland soil is driven by long-term climate warming

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Anthropogenic climate change threatens ecosystem functioning. Soil biodiversity is essential for maintaining the health of terrestrial systems, but how climate change affects the richness and abundance of soil microbial communities remains unresolved. We examined the effects of warming, altered precipitation and annual biomass removal on grassland soil bacterial, fungal and protistan communities over 7 years to determine how these representative climate changes impact microbial biodiversity and ecosystem functioning. We show that experimental warming and the concomitant reductions in soil moisture play a predominant role in shaping microbial biodiversity by decreasing the richness of bacteria (9.6%), fungi (14.5%) and protists (7.5%). Our results also show positive associations between microbial biodiversity and ecosystem functional processes, such as gross primary productivity and microbial biomass. We conclude that the detrimental effects of biodiversity loss might be more severe in a warmer world.

Biodiversity, the variety of genes, species and ecosystems that constitute life on our planet[1], is dramatically affected by human alterations of the global environment[2]. Biodiversity underscores healthy ecosystem functions and assures the production of essential goods, services and benefits to society, such as climate regulation, landscape stability, fibres and food production[1]. However, such benefits are threatened by the unprecedented biodiversity loss[3,4] caused by anthropogenic global environmental changes such as climate warming, altered precipitation patterns and land-use changes[5]. Studies demonstrate that biodiversity loss impairs the functioning of natural ecosystems and diminishes the number and quality of services they provide[6]. Thus, it is imperative to understand how global environmental change affects biodiversity and the underlying mechanisms[7].

Anthropogenic climate changes are the greatest threats to biodiversity from local to global scales[5,6]. The effects of climate change on biodiversity include shifts in species' geographical ranges[4], extinction[8–10], changes in abundance within species ranges[11], loss of phylogenetic community diversity[12], and increased genetic mutation and selection[13]. In contrast to plants and animals, our understanding of climate change effects on microbial biodiversity are poorly understood. Previous studies have demonstrated the effects of climate warming on soil microbial communities in terms of respiratory feedback responses[14,15], decomposition[16], microbial biomass[17], community composition[14,15,18,19], community succession[18], temporal scaling[19], and network complexity and stability[20]. However, there is a paucity of information on the effects of warming on below-ground microbial biodiversity (that is, alpha diversity) due to the lack of well-replicated, long-term time-series observations under realistic field settings that is necessary to discern clear warming impacts. Therefore, despite a longstanding interest in this topic, whether and how climate warming would result in net soil microbial biodiversity gain or loss, and their underlying mechanisms remain unresolved.

Because different species differ greatly in their temperature-dependent metabolic rates, rising temperature would have dramatic effects on resource consumption, growth, reproduction and interactions between species (for example, competition, predation, parasitism and symbiosis)[9]. On one hand, certain species with higher fitness at elevated temperature will probably have a competitive advantage over other species that are less fit[21]. Consequently, warming could trigger extinction events at local scales and drive biodiversity loss, which may further cause extinction of other species through coextinction cascades[8]. Similarly, warming and associated environmental changes, such as decreased moisture, would act as strong filtering factors against existing microbial species, which could also cause biodiversity loss. On the other hand, in general, warming promotes plant productivity[14,15]. Such potentially higher plant diversity and/or quantity of resources could support more microbial species by providing more niches with more ways for species to coexist[21], and result in biodiversity gain. In addition, the effects of these factors could be intertwined, resulting in no change in biodiversity.

To determine whether and how climate warming affects soil biodiversity, we examined the taxonomic and phylogenetic diversity

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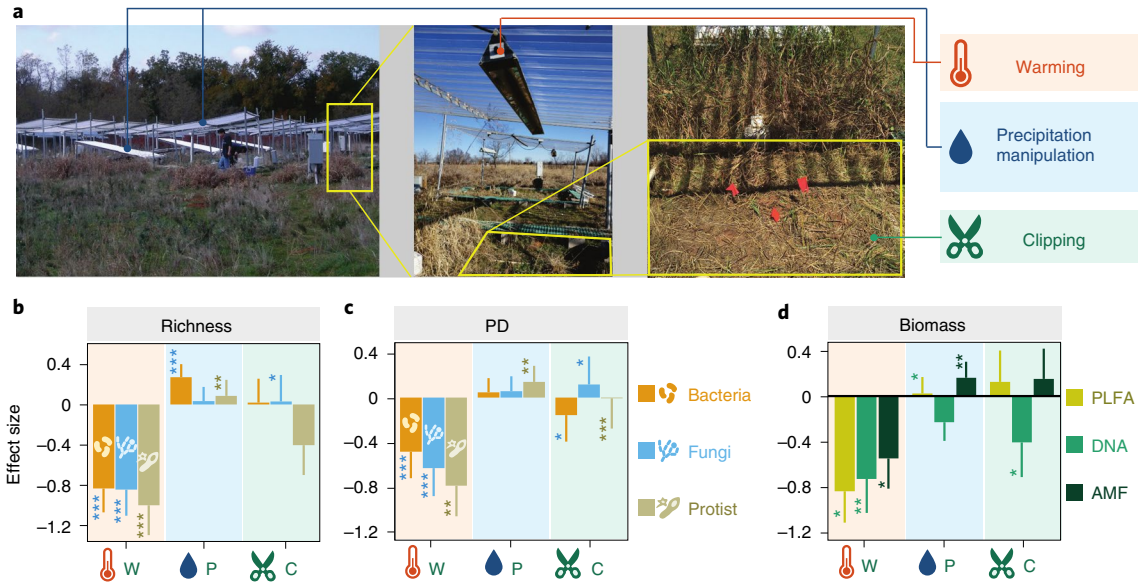

Fig. 1 | Effects of experimental warming on soil microbial communities. a, Experimental settings for treatments. **b**–**d**, The effect sizes of warming, altered precipitation level and clipping on microbial richness (**b**), phylogenetic diversity (**c**) and biomass (**d**). The estimated effect sizes are regression coefficients based on rescaled response variables (with zero mean and unit standard deviation) in the linear mixed-effects models. Bars represent mean ± s.e.m. of effect sizes. The effects of treatment interactions and exact *P* values are indicated in Supplementary Tables 1 and 2. Statistical significance is based on Wald type II χ^2 tests ($n = 360$); ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. PD, Faith's phylogenetic diversity.

(PD) of grassland soil bacteria, fungi and protists in a multifactor global change experiment[19] over 7 consecutive years from 2009 to 2016. We used warming (+3 °C) and altered precipitation levels (that is, −50% of ambient precipitation or half precipitation, and +100% or double precipitation) as primary factors, and clipping (annual removal of above-ground biomass to simulate the land-use practice of mowing for hay[22] or bioenergy[23]) as a secondary factor. We address the following major questions: whether and how experimental warming, altered precipitation and clipping affect soil microbial biodiversity over time; whether such effects vary among different microbial lineages; and what are their underlying mechanisms. We hypothesize that warming would reduce the biodiversity of soil bacteria, fungi and protists via alteration of both environmental filtering and biotic interactions.

Results

Effects of climate change factors on soil and plant variables. Linear mixed-effects models (LMMs) for determining the sources of variations in hierarchical biological data were first employed to test the effects of treatments and their interactions on soil biogeochemistry and plant communities. In these models, the regression coefficients represent the directions and magnitudes of the treatment effects, namely effect sizes (β). By comparing the β values, our results revealed that experimental warming, compared with altered precipitation levels and clipping (Fig. 1a and Extended Data Fig. 1), had predominant effects on soil microclimate by increasing temperature but decreasing moisture (Extended Data Fig. 2a,b and Supplementary Note A), and on geochemistry (for example, decreasing soil pH, increasing NO_3^-) (Extended Data Fig. 2c,d and Supplementary Note A). For example, on average, warming decreased soil moisture by 1.5% (absolute) ($\beta = -1.5$, $P < 0.0001$; Extended Data Fig. 2b). In comparison, half precipitation only decreased soil moisture by 0.35%, while double precipitation increased soil moisture by 0.7% ($\beta = 0.7$, $P < 0.0001$; Extended Data Fig. 2b). As expected, clipping had significant negative effect on plant biomass, but positive effect on plant richness (Extended Data Fig. 2f,g and Supplementary Note A).

Impacts of warming on microbial biodiversity. It is expected that the alterations in soil microclimate, geochemistry and plant communities would lead to changes in soil microbial biodiversity. Here we define biodiversity[21] as taxonomic (that is, species richness and their relative abundance) and phylogenetic[19] diversity in a local community. To test this prediction, all samples were analysed for bacteria (56,182 ± 27,613 reads per sample), fungi (23,569 ± 16,323 reads per sample) and protists (11,146 ± 10,528 reads per sample) (Extended Data Figs. 3 and 4). Linear mixed-effects models revealed that warming had strong negative effects ($\beta = -0.84$ to -0.11, $P < 0.007$) on richness and other taxonomic diversity indices, and on Faith's phylogenetic diversity, which is the sum of the total phylogenetic branch lengths based on the phylogenetic tree constructed (Fig. 1b–f, and Supplementary Table 1 and Note B1). In general, although precipitation alteration or clipping could exert significant effects on richness (for example, precipitation alteration on bacterial richness; Fig. 1b), the effect sizes of warming on richness were 3–41 times larger than those of other treatments (Fig. 1b, and Supplementary Table 1 and Note B1). In addition, the effects of treatment interactions were rarely significant except for the positive interactive effects of warming and clipping on fungal and protistan diversity ($\beta = 0.08$–0.91, $P < 0.05$) (Supplementary Table 1), indicating that the warming effect was largely independent of altered precipitation and clipping. Collectively, these results suggest that the diversity of soil bacteria, fungi and protists is predominantly shaped by experimental warming. A possible explanation is that the changes in microbial biodiversity are mainly driven by soil microclimate and geochemistry such as soil temperature, moisture and pH[24–26]. As shown above, experimental warming had larger effects on these variables compared with the effects of the half/double precipitation and clipping treatments. Therefore, we will primarily focus on warming-induced treatment effects in subsequent sections.

Overall, warming significantly reduced bacterial richness by 9.6% ($\beta = -0.83$, $P < 0.0001$), fungal richness by 14.5% ($\beta = -0.84$, $P < 0.0001$) and protistan richness by 7.5% ($\beta = -0.99$, $P < 0.0001$). Such negative effects varied yearly with significant impacts on bacteria ($\beta = -1.72$ to -0.68, $P < 0.05$) after 2011, fungi ($\beta = -2.15$ to

−0.36, $P<0.05$) after 2013, and protists in 2011, 2013 and 2014 ($\beta=-1.44$ to -0.60, $P<0.05$) (Extended Data Fig. 5 and Supplementary Note B2). Rarefaction analyses indicated that the observed richness for bacteria, fungi and protists were always lower under warming than non-warming control, except warming & double precipitation & clipping (WDC) versus double precipitation & clipping (DC) for fungi, and warming & clipping (WC) versus clipping (C) for protists (paired *t*-test, $P<0.0001$; Extended Data Fig. 4). Warming also significantly decreased the phylogenetic diversity of bacteria by 7.2% ($\beta=-0.49$, $P<0.0001$), fungi by 9.3% ($\beta=-0.47$, $P=0.002$) and protists by 4.5% ($\beta=-0.80$, $P=0.003$) on the basis of Faith's PD, the phylogenetic analogue of taxon richness (Fig. 1f and Supplementary Table 1). In addition, consistent with warming-induced biodiversity decrease, warming significantly reduced microbial biomass as determined by phospholipid fatty acid analysis (PLFA) ($\beta=-0.83$, $P=0.046$) and DNA yields ($\beta=-0.72$, $P=0.002$; Fig. 1g, and Supplementary Table 2 and Note B1). Collectively, all of these results indicate that experimental warming significantly reduced microbial biodiversity.

The negative warming effects on microbial biodiversity varied considerably among different microbial lineages. Warming significantly decreased the richness of most microbial phyla (Fig. 2a), as well as their phylogenetic diversity (Extended Data Fig. 6a and Supplementary Note C1). Warming had largest negative effects on the richness of Acidobacteria, Verrucomicrobia and Planctomycetes ($\beta=-1.21$ to -1.19, $P<0.01$), but had a significant positive effect on the richness of Firmicutes ($\beta=1.52$, $P<0.01$; Fig. 2a). Similar to species richness, warming significantly decreased the relative abundance of Acidobacteria, Verrucomicrobia and Planctomycetes ($\beta=-0.88$ to -0.84, $P<0.01$). In contrast, warming increased the relative abundance of Actinobacteria, Firmicutes and Gemmatimonadetes ($\beta=0.52$–1.05, $P<0.05$; Extended Data Fig. 6b), which could be due to their preference for drier soils[27–29]. Notably, the increase of Firmicutes and Actinobacteria may in part be due to their spore-forming ability[30], which makes them resistant to desiccation stress. In support of this, we examined the characteristics of spore-forming bacteria in more detail. Almost all the families of Firmicutes and Actinobacteria that were increased under warming are known spore-formers[30] (Extended Data Fig. 7a,b). In addition, the relative abundances of the major sporulation genes in Firmicutes (spo0A) and Actinobacteria (bldD), as identified from shotgun sequencing metagenome data, also significantly or marginally significantly increased under warming ($P<0.0001$ for bldD; $P=0.08$ for spo0A; Extended Data Fig. 7c). Similar patterns were also observed for individual amplicon sequence variant (ASV) levels (Fig. 2b). For instance, most taxa in Verrucomicrobia (78.5% of ASVs of Verrucomicrobia, 91.8% of relative abundance) decreased under warming, while most taxa in Firmicutes (88.0% of ASVs, 98.9% of relative abundance) increased (Fig. 2b and Supplementary Note C2). In addition, warming effects varied among different fungal guilds as classified by FUNGuild[31]. Warming reduced the richness, phylogenetic diversity and abundance of arbuscular mycorrhiza fungi (AMF) ($\beta=-1.05$ to -0.42, $P<0.01$; Fig. 2a, Extended Data Fig. 6a,b and Supplementary Note D1), which are beneficial microorganisms capable of forming mutualistic symbiosis with plants. The negative warming effect on AMF abundance was also supported by AMF biomass decreases as determined by PLFA ($\beta=-0.54$, $P=0.013$; Fig. 1g). Interestingly, although warming decreased the richness of putative plant pathogenic fungi (Fig. 2a), it marginally increased their relative abundance ($\beta=0.43$, $P=0.055$; Extended Data Fig. 6b), which could have negative effects on plant growth. Moreover, warming significantly reduced the richness, phylogenetic diversity and abundance of Cerozoa and Ochrophyta ($\beta=-1.07$ to -0.20, $P<0.002$) but increased the richness and phylogenetic diversity of Conosa ($\beta=0.05$–0.12, $P<0.02$) (Fig. 2a, Extended Data Fig. 6a,b and Supplementary Note C1). Similarly, warming significantly decreased the richness and phylogenetic diversity of various functional groups of protists (that is, consumers, phototrophs and parasites) ($\beta=-0.98$ to -0.39, $P<0.04$). Warming also reduced the relative abundance of phototrophic protists ($\beta=-0.17$, $P=0.01$) (Fig. 2a and Extended Data Fig. 6a,b). These results suggest that warming has differential impacts on various microbial lineages and/or functional guilds, which are consistent with our previous observations that warming effects vary greatly among different microbial functional groups[15]. Warming-induced diversity decrease on most microbial categories could have significant impacts on ecosystem functioning, as suggested by previous reports in macroecology[3,4,6] and microbial ecology[32]. Particularly, since warming decreased beneficial taxa such as AMF, the above-ground plant community could be negatively impacted.

Mechanisms underlying reduced microbial biodiversity. As we posited earlier, warming-induced biodiversity decrease could be due to changes in biotic interactions and abiotic environmental conditions caused by warming. Under warmer conditions, many microbes with adaptive traits (for example, Firmicutes and Actinobacteria with spore-forming ability) would survive and outcompete other microbes (for example, Acidobacteria, Verrucomicrobia and Planctomycetes) (Fig. 2a,b). Consequently, species coexistence patterns would be substantially altered, as revealed by a network analysis showing that the occurrence network was more complex under warming than the non-warming control[20]. The increased positive connections may indicate more microbial cooperations[33], which could be important for their survival under warming. Also, there were more negative connections under warming than in the control[20], suggesting that there might be more intense competition under warming. Eventually, the warming-induced changes in microbial activities and interactions could trigger various extinction events and ultimate biodiversity decrease due to cascading effects[8]. Alternatively, warming could just act as a deterministic filtering factor to impose significant positive selection on spore-forming microorganisms (for example, Bacillaceae_2) and/or negative selection on non-spore-forming microorganisms (for example Acidothermaceae), which is consistent with the observation that warming enhanced homogeneous selection on Bacillales in Firmicutes[34]. All of these results suggest that both biotic interactions and environmental filtering could play important roles in mediating warming-induced biodiversity decrease.

It is anticipated that soil environmental conditions would also play important roles in driving microbial biodiversity decrease. As shown in Fig. 3a, bacterial, fungal and protistan richness were highly correlated with soil moisture, temperature and NO_3^--N content (LMMs $r=-0.25$–0.24, $P<0.01$). Bacterial richness also showed significant correlations with plant richness and biomass (LMMs $r=0.11$–0.19, $P<0.05$; Supplementary Note D1). However, obvious collinearity among these variables also occurred (Fig. 3a and Supplementary Note D1). Thus, to further disentangle the direct and indirect effects of the environmental drivers on microbial biodiversity, structural equation modelling (SEM) analyses were performed with the presumed relationships (Extended Data Fig. 8) among the selected subsets of plant and soil variables that were least correlated (see Methods for details of model selection). Soil moisture, which was negatively affected by warming (standardized path coefficient, $b=-0.69$) and half precipitation ($b=-0.16$), but positively affected by double precipitation ($b=0.45$), played the strongest role in directly shaping bacterial richness ($b=0.43$, $P=0.001$; Fig. 3b and Supplementary Note D2). Soil pH, plant richness and the biomass of C_3 plants were also significantly and positively ($b=0.23$–0.31, $P<0.02$) correlated with bacterial richness. Furthermore, bacterial richness directly and positively affected protistan richness ($b=0.69$, $P<0.001$). In comparison, among the variables that directly contributed to fungal richness,

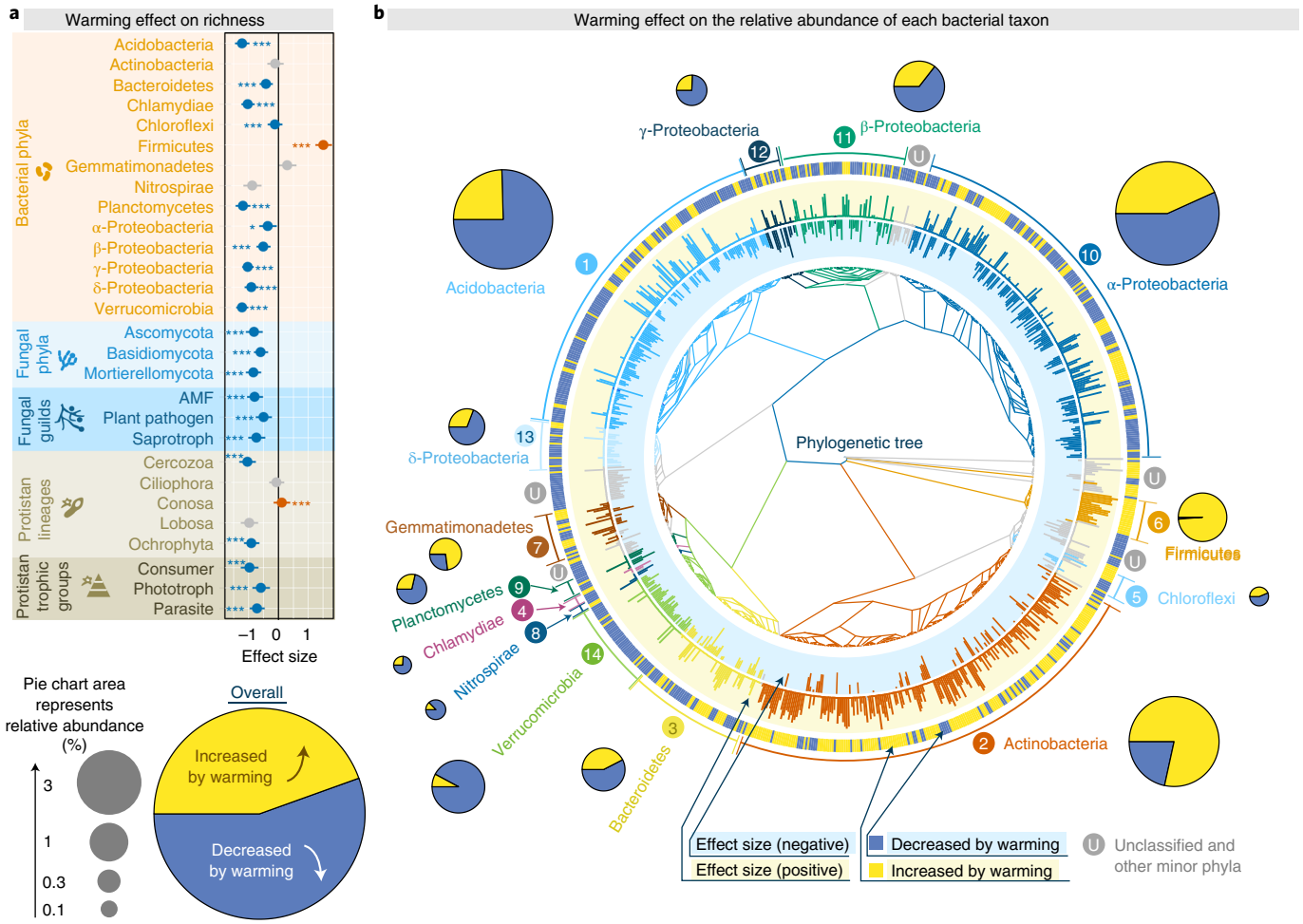

Fig. 2 | Effects of experimental warming on different microbial taxa. a, Effect sizes of warming on the (rescaled) richness of major microbial groups based on linear mixed-effects models. Data are presented as mean ± s.e.m. of the estimated effect sizes. Statistical significance is based on Wald type II χ^2 tests ($n = 360$); $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. Non-significant changes are denoted by grey dots. **b**, The phylogenetic relationships of individual bacterial ASVs (the innermost ring). Only ASVs with a significant response (adjusted $P < 0.05$) to warming and with average read numbers ≥2 among warmed or unwarmed samples were included in the tree. The outside and inside bars of the second ring represent the positive and negative effect sizes of warming on rescaled taxon relative abundances. Colours of the branches in the first ring and the bars in the second ring correspond to individual phyla or classes, and the grey colour in the tree indicates unclassified or other minor phyla. Colours in the third ring represent ASVs with significant increase (yellow) or decrease (blue) under warming. The area sizes of the pies reflect the total relative abundance of bacterial phyla/classes across all samples, where the yellow and blue parts represent the proportions of the total abundance of ASVs that increased and decreased under warming, respectively.

only paths of soil moisture ($b = 0.44$, $P = 0.001$) and plant richness ($b = 0.26$, $P = 0.015$) were significant (Extended Data Fig. 9), suggesting that the environmental drivers appear different between bacteria and fungi. Overall, these variables can explain 61%, 51% and 50% of the variations in bacterial, fungal and protistan richness (Fig. 3b and Extended Data Fig. 9), respectively. In addition, SEM analysis revealed that warming played predominant roles in shaping microbial diversity (standardized total coefficient = −0.61 for bacteria, −0.56 for fungi and −0.51 for protists; Fig. 3c) compared with precipitation or clipping treatments (standardized total coefficients = −0.05–0.31; Fig. 3c), which was consistent with linear mixed-effects model analysis (Fig. 1a). These results indicate that soil and plant variables, particularly soil moisture, are also important in mediating warming-induced soil microbial diversity decrease both directly and indirectly. Since the SEMs could explain over half of the variations in microbial diversity, the environmental filtering effects, especially the induced desiccation stress, could be the main driver for microbial diversity decrease via affecting microbial activities and interactions.

Links between microbial biodiversity and ecosystem functions. An important follow-up question is whether the warming-induced changes in microbial diversity affect ecosystem functional processes. Consistent with the reduced microbial biodiversity, warming also decreased the ecosystem functions of total microbial biomass, bacterial biomass, gross primary productivity (GPP) and ecosystem respiration (ER) ($\beta = -0.17$ to -0.84; Extended Data Fig. 10). In agreement with various reports in macroecology[6], the overall bacterial richness had significant positive correlations with total microbial biomass, bacterial biomass, GPP and ER ($r = 0.14$–0.22, $P < 0.002$) (Fig. 3d and Supplementary Note E). Similar positive correlation patterns were also observed for most bacterial groups (for example, Proteobacteria, Bacteroidetes, Planctomycetes) (Fig. 3d and Supplementary Note E) except for Firmicutes, which showed significant negative correlations with total microbial biomass, bacterial and fungal biomass, and ER ($r = -0.26$ to -0.10, $P < 0.04$). In addition, the overall richness of fungi and most fungal phyla/guilds showed significant positive correlations with GPP and ER (Fig. 3d and Supplementary

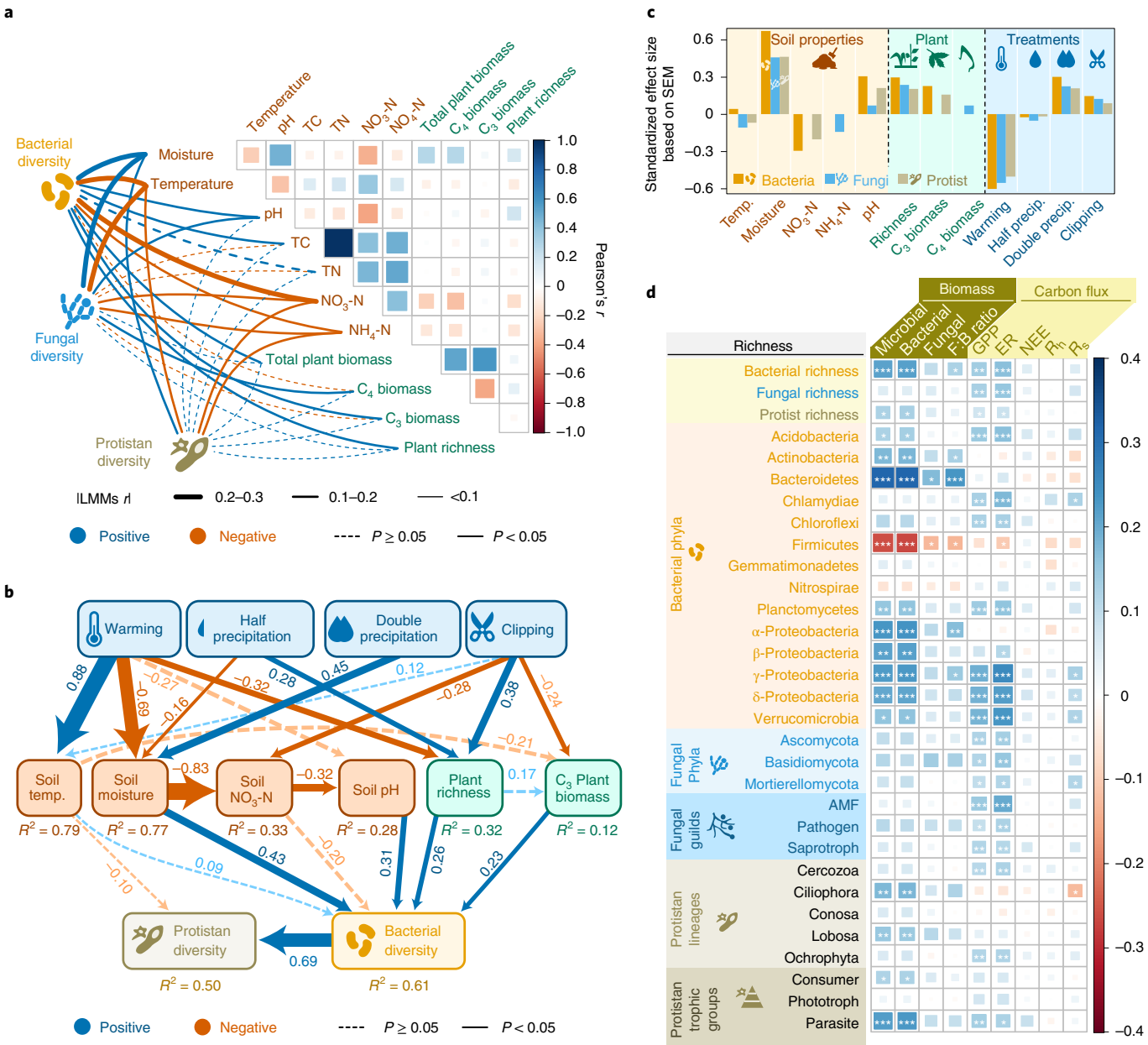

Fig. 3 | Environmental drivers of microbial diversity. a, Correlations between environmental variables and microbial diversity. Edge width corresponds to the absolute value of the correlation coefficient determined by the linear mixed-effects models. Colours indicate correlation types. Solid and dashed lines denote significant and non-significant correlations, respectively, based on Wald type II χ^2 tests ($n = 360$ biologically independent soil samples). Pairwise comparisons of environmental factors are shown in the triangle, with a colour gradient denoting Pearson's correlation coefficient. **b**, Structural equation models (SEMs) showing the relationships among treatments, soil and plant variables, and bacterial and protistan richness. Blue and red arrows indicate positive and negative relationships, respectively. Solid or dashed lines indicate significant ($P < 0.05$) or non-significant relationships. Numbers near the pathway arrow indicate the standard path coefficients. R^2 represents the proportion of variance explained for every dependent variable. $\chi^2 = 47.69$, d.f. = 34, $P = 0.06$ (large P value indicates that the predicted model and observed data are equal, that is, good model fitting). Comparative fit index (CFI) = 0.955, and $n = 48$ independent plots. **c**, Standardized total effects (direct plus indirect effects) derived from SEMs. **d**, Correlations between microbial richness and ecosystem functioning. The colour denotes the correlation coefficient determined by the linear mixed-effects model. Statistical significance is based on Wald type II χ^2 tests with $n = 360$ independent soil samples. The P values were adjusted by false discovery rate; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Note E). The overall protistan richness, major protistan lineages and functional groups also had significant positive correlations with total microbial biomass, bacterial biomass, GPP and ER ($r = 0.08$–0.22, $P < 0.04$) (Fig. 3d and Supplementary Note E). All of these results indicate that there are significant positive linkages between microbial community diversity and relevant ecosystem functional processes.

Discussion

Understanding how climate change affects microbes and the underlying mechanisms is a critical issue in climate change and microbiology research[35]. By examining the dynamic changes in soil microbial biodiversity in a well-replicated long-term climate change experiment, this study provides explicit evidence that climate warming consistently reduced the taxonomic and phylogenetic diversity of

soil bacteria, fungi and protists across different years. In addition, by examining the interactive effects of warming, precipitation level and clipping on microbial diversity, this study demonstrates that warming plays a predominant role in driving soil biodiversity decrease via altering biotic interactions and soil biogeochemical conditions, particularly soil moisture, which is in agreement with the fact that warming had prevalent effects on accelerating the temporal scaling rates of soil microbial biodiversity[19]. Finally, warming-induced diversity decrease could have significant impacts on ecosystem functioning, which augments previous reports in macroecology[3,4,6].

Our findings have important implications for predicting ecological consequences of climate change and for ecosystem management. Because warming as a deterministic filtering factor drives microbial biodiversity decrease[18], the ecosystems under future climate change scenarios would be less diverse. Along with faster biodiversity turnover rates as previously demonstrated[19], it is expected that the linked ecosystem functions and services could become more vulnerable in a warmer world[6]. Particularly, since warming has differential effects on different microbial lineages, such as the decrease of beneficial taxa (for example, AMF), the detrimental effects of biodiversity loss on future ecosystem functioning could be more severe. In addition, since warming effects on biodiversity are primarily via reduced moisture, it is expected that warming-induced biodiversity decrease would be more severe in drylands (that is, arid, semi-arid and dry-subhumid ecosystems), covering 41% of Earth's land[36], as compared with wet regions. The future warming-induced precipitation changes could also be important in mediating warming-induced biodiversity decrease. However, further research is necessary to determine whether the warming-induced biodiversity decrease and associated mechanisms are applicable to other ecosystems.

Methods

Study site and sampling. We conducted the warming experiment at the Kessler Atmospheric and Ecological Field Station (KAEFS) in the US Great Plains in McClain County, Oklahoma (34° 59′N, 97° 31′W)[18–20,22]. Detailed site description can be found in Supplementary Note F. In brief, KAEFS is an old-field tall-grass prairie with dominant plants of C_3 forbs (Ambrosia trifida, Solanum carolinense and Euphorbia dentate) and C_4 grasses (Sorghum halepense and Tridens flavus)[22]. Based on Oklahoma Climatological Survey data from 1948 to 1999, the air temperature ranges from 3.3 °C in January to 28.1 °C in July with mean annual temperature 16.3 °C, and the precipitation ranges from 82 mm in January and February to 240 mm in May and June with mean annual precipitation 914 mm[15]. The soil type of this site is Port–Pulaski–Keokuk complex, and soil texture class is loam with 51% sand, 35% silt and 13% clay[37].

The field site experiment was established in July 2009 with a blocked split-plot design (Extended Data Fig. 1) in which warming (continuous heating at a target of +3 °C above ambient temperature) and precipitation alteration (targets of −50% and +100% of ambient precipitation) were primary factors nested with clipping (annual removal of above-ground biomass at peak growth season) as the secondary factor[18–20]. The clipping treatment was used to mimic the land-use practice of hay harvest, which is widely practiced in the southern Great Plains of the USA[22], and biomass harvest for bioenergy[23]. In brief, the site has four experimental blocks, each including six plots. Each plot has a size of 2.5 × 3.5 m^2, which was further divided into one 2.5 × 1.75 m^2 clipped subplot and one 2.5 × 1.75 m^2 unclipped subplot, resulting in a total of 48 subplots (Extended Data Fig. 1).

From 2009 to 2016, surface (0–15 cm) soil samples were collected annually from subplots one day before annual clipping. Each sample was mixed from three soil cores (2.5 cm diameter × 15 cm depth) from a soil sampler tube. In the first year (2009), we collected 24 pre-warmed soil samples from the southern subplots. In subsequent years, a total of 48 annual soil samples were collected from all subplots in each year. A total of 360 annual soil samples from 2009 to 2016 were collected in this study and stored in a freezer at −80 °C.

Field measurements and soil chemical analyses. Soil temperature was measured every 15 min at depths of 7.5, 20, 45 and 75 cm in the centre of each plot using constantan-copper thermocouples wired to a CR10x data logger (Campbell Scientific)[18–20]. Annual average values of temperature at a depth 7.5 cm were used to represent soil temperature across experimental years. Volumetric soil water content (%V) was measured using a portable time-domain reflectometer (Soil Moisture Equipment) once or twice a month and annual average values were used to represent soil moisture[18–20]. Ecosystem carbon (C) fluxes, including ecosystem respiration (ER), net ecosystem exchange (NEE), soil total respiration (R_s) and heterotrophic respiration (R_h) were measured once or twice a month between 10:00 and 15:00 (local time)[18–20]. The GPP was then estimated as the difference between net ecosystem exchange and ecosystem respiration.

Above-ground plant community surveys were conducted at peak biomass (usually September) each year. All species within each plot were identified to estimate species richness. From 2009 to 2015, the above-ground plant biomass, separated into C_3 and C_4 species, was estimated by a modified pin-touch method, as previously described[38]. Since 2016, a thorough plant survey was conducted and linear regression models were used to estimate above-ground biomass on the basis of plant height and abundance[39]. Every individual plant in the whole plot was surveyed, with their species identity and height recorded. For each species, we also measured the height across different individuals off-plot, after which we harvested, dried and weighed the vegetation which we had recorded. We then constructed regression relationships between plant height and dry weight biomass for each species. The regression model for one species, *Pseudognaphalium obtusifolium* was non-significant ($R^2 = 0.07$, $P = 0.48$), and we used the mean biomass across individuals collected off-plot as the biomass estimate for its individual in the plot. The average adjusted R^2 for all other regression models was 0.76 and the average P value was 0.0056. The total above-ground biomass was then calculated as the sum of estimated biomass for every individual.

Visible stones and plant roots were removed from the soil by metal forceps before chemical and microbial analyses. The chemical properties of all soil samples were analysed in the Soil, Water and Forage Analytical Laboratory at Oklahoma State University. Briefly, the total C and total nitrogen (N) contents were determined using a dry combustion C and N analyser (LECO). Soil nitrate (NO_3^-) and ammonia (NH_4^+) were analysed using a Lachat 8000 flow-injection analyser (Lachat). Soil pH was determined using a pH meter with a calibrated combined glass electrode at a water-to-soil mass ratio of 2.5:1[40].

PLFA. Lipids were extracted from the soil samples on the basis of the modified Bligh-Dyer method as previously described[41]. In brief, soil samples were freeze dried and sifted to remove any rocks or large debris. Each freeze-dried soil sample (2 g) was then incubated in a 2:1:0.8 solution of methanol, chloroform and K_2HPO_4 buffer. The chloroform phases were collected and the phospholipids were separated from neutral lipids and glycolipids through silicic acid chromatography, subsequently saponified and methylated to fatty-acid methyl esters. The resulting fatty-acid methyl esters were separated and identified using gas chromatography (Agilent 6890N). The peak responses were translated into molar responses using an internal standard and were fitted with a MIDI Sherlock microbial identification system (version 4.5, MIDI). Further, the peak responses were assigned to microbial groups including Gram-negative bacteria, Gram-positive bacteria, Actinobacteria, anaerobic bacteria, common fungi and AMF using the Agilent Chemstation software (Agilent Technologies). The total bacterial biomass of the soils was calculated as the total PLFA of all bacterial groups, that is, the sum of the biomass of Gram-negative bacteria, Gram-positive bacteria, Actinobacteria and anaerobic bacteria. The total fungal biomass was calculated as the sum of the biomass of common fungi and AMF.

DNA extraction. It is well known that sequence quality is subjected to wide variations from DNA extraction, PCR amplification and sequencing. It is critical to control each step to generate high-quality experimental data. Thus, great caution was taken in this study to ensure highest quality sequencing data with more tedious laboratory experimental protocols. For DNA extraction, the experimental method with grinding, freeze-thawing and sodium dodecyl sulfate (SDS)-based cell lysis[42] was used. This method has been most widely used in microbial molecular ecology. In brief, for each soil sample, microbial DNA was extracted from 1.5 g soil using this grinding, freeze-thawing method[42], and purified with a PowerSoil DNA isolation kit (MoBio Laboratories) following the manufacturer's protocol. DNA quality was evaluated on the basis of the 260/280 nm and 260/230 nm absorbance ratios using an ND-1000 spectrophotometer (NanoDrop Technologies). All samples had 260/230 ratios >1.7 and 260/280 ratios >1.8. DNA concentration was measured by PicoGreen using a FLUOstar Optima fluorescence plant reader (BMG Labtech). DNA samples were stored at −80 °C until use.

Amplicon sequencing and data preprocessing. We used a two-step PCR amplification method for library preparation of the 16S rRNA gene (V4 region), the intergenic region (ITS) between the 5.8S and 28S rRNA genes, and the 18S rRNA gene (V9 region) to improve sequence representation and quantification[43,44]. During the first amplification step, 10 ng DNA from each sample was PCR-amplified for 10 cycles in triplicate in a 25 µl reaction volume with the primers without adaptors. The obtained PCR products were purified and dissolved in 50 µl deionised water. This initial amplification step avoided potential amplification bias caused by long tails of adaptors and other added components. During the second amplification step, 15 µl of the PCR products from each sample were amplified using the primers with all adaptor, barcode and spacers in triplicate for an additional 15 cycles. The low total cycle numbers (25–30 cycles) ensure that the PCR amplification is not saturated and also limit amplification artefacts. Finally, the triplicate amplified products were combined, purified and quantified for subsequent sequencing using the same MiSeq instrument with 2 × 250 base pair kits at the Institute for Environmental Genomics, University of Oklahoma. The two-step PCR amplification method with phasing primers in

triplicate can help reduce sequencing errors, minimize amplification bias and preserve semi-quantitative information of PCR amplification[43], which is critical for subsequent data analysis, data interpretation and biological inference[44].

The primer sequences were trimmed from the paired-end sequences, which were then merged using FLASH[45]. Any merged sequences with an ambiguous base or a length of <245 bp for the 16S rRNA gene, <220 bp for the ITS, or <330 bp for the 18S rRNA gene were further discarded. An average of 56,182 ± 27,613, 23,569 ± 16,323 and 56,874 ± 55,642 sequence reads were obtained for the 16S rRNA gene, ITS and 18S rRNA genes, respectively (Extended Data Fig. 3a,b). These high-quality 16S rRNA gene, ITS or 18S rRNA gene sequences were processed to generate amplicon sequence variants (ASVs; also known as unique sequence variants and zero-radius operational taxonomic units (OTUs)) by UNOISE3[46]. Rarefaction analyses (Extended Data Fig. 4) indicated that the sequencing depth was sufficient for assessing the effects of various climate change factors on the diversity of these soil microbial communities.

The representative 16S rRNA or 18 rRNA gene sequences were aligned using Clustal Omega v1.2.2[47] for constructing the phylogenetic tree by FastTree2 v2.1.10[48]. The FastTree topology search was constrained with the relatively reliable 16S-based bacterial tree in Silva Living Tree Project[49] release 132. The fungal phylogenetic tree was constructed using 'constrained topology search' in FastTree v2.1.11[50]. A guide tree was built from the full-length small subunit rRNA sequences of 511 representative species, one species from each fungal family. Then, the full tree was built from the observed ITS sequences with the constraint alignment converted from the guide tree. The small subunit rRNA sequences were retrieved from Silva 138.1 Ref NR database. For 16S rRNA genes, the ASVs or OTUs were taxonomically annotated with RDP Classifier using 16S rRNA gene training set 16 with a confidence cut-off of 50%[51], and chloroplast and mitochondria were further removed from the bacterial profiles. For ITS, the ASVs or OTUs were taxonomically annotated with RDP Classifier using UNITE Fungal ITS training set (version of August 2018)[52]; ITS sequences were further assigned into three functional groups—plant pathogens, AMF and saprotrophs using FUNGuild[31]. Sequences that had multiple function assignments in FUNGuild were termed as unassigned guild in this analysis. The sequence number in each sample was rarefied to the same depth for the 16S rRNA gene (22,599) or ITS sequences (7,761) in subsequent comparative analyses. For the 18S rRNA gene, the ASVs were taxonomically annotated with PR2 databases[53]. We also assigned the major protistan lineages to their dominant mode of energy acquisition (that is, trophic functional groups)—either phototrophic, parasitic or as consumers, following the classification in Oliverio et al.[54]. Sequences annotated as protists were further rarefied to 1,100 for subsequent comparative analyses.

Sporulation gene profiling from metagenomic dataset. The soil samples under single treatment of warming (warming and normal precipitation and unclipped) and control (ambient temperature and normal precipitation and unclipped) were selected for metagenomic sequencing. That is, 8 subplots × 8 years = 64 metagenomic samples. Libraries were constructed using genomic DNA with KAPA Hyper Prep kit (KR0961) following the manufacturer's instruction, and DNA was sequenced using an Illumina HiSeq 2500 platform. A total of 1,100.14 gigabases (Gb) were generated, with an average of 17.19 ± 2.68 Gb per sample. The quality of the metagenomic data was evaluated using FastQC v0.11.6[55]. CD-HIT[56] was used to remove duplicates with an identity cut-off of 100%. NGS QC Toolkit (version 2.3.3)[57] was used for quality filtering, where poor-quality bases with quality score <20 were trimmed from the 3' end until the first base had a quality score ≥20. Trimmed reads with length of >120 and average quality score ≥20 were kept. In addition, reads with more than one ambiguous base were removed. High-quality reads were then converted to fasta format, split into multiple partitions and searched against the NR database (BLASTx) using DIAMOND[58] with E value cut-off of 1×10^{-5}, coverage cut-off of 0.5 and maximum target number of 50. The outputs were submitted to MEGAN6 (Ultimate Edition, version 6.6)[59] for function profiling, with parameter of top percent of hits 10%, minimum score of 50 and minimum support of 1. The annotated functional profiles of the SEED Subsystem (3 levels) were exported and two major sporulation genes annotated at level 3, that is, spo0A gene of Firmicutes and bldD gene of Actinobacteria were selected. Their relative abundances were then determined by dividing the annotated sequence counts by the total number of high-quality sequences of the corresponding metagenomes.

Statistical analyses. This study is based on a well-designed long-term climate change experiment with a blocked split-plot design, with 12 treatment combinations of warming, precipitation levels and clipping. Each combination has 4 replicated plots (Extended Data Fig. 1). Also, the same plots were repeatedly sampled over 8 years and high-quality experimental data were generated, which greatly increased the power for various robust data analyses to ensure the reliability of the statistical inference.

Diversity analyses. Richness was used to measure taxonomic α-diversity, using the 'Picante' R package[60,61]. Other taxonomic α-diversity indices, including Shannon index, inverse Simpson index and Pielou's evenness were also calculated using the 'vegan' R package[62]. Faith's index was used to measure phylogenetic α-diversity using the Picante R package[60].

Treatment effects by LMMs. Due to the block design and repeated measurements, the experimental data are not completely independent. Therefore, LMMs were used to assess the effects of experimental treatments on environmental variables, microbial diversity or the relative abundance of microbial groups. The lme4 R package was used to implement LMMs[63]. In the LMMs, warming (0 for ambient temperature and 1 for warming), precipitation level (0.5 for half, 1 for normal and 2 for double precipitation level) and clipping (0 for unclipped and 1 for clipped) treatments and their interactions were considered as fixed effects, while sampling time (year) and block were termed as random intercept effects (y ~ warming × precipitation level × clipping + (1 | block) + (1 | year)). That is, three variables (warming, precipitation level and clipping) were created to denote the experimental treatments for soil samples. The reason that the precipitation treatments were not treated as categorical variables is because a categorical variable cannot capture the gradient of precipitation levels, which is assumed to linearly correlate with soil moisture and other variables.

We also tested alternative models in which the effects of sampling year and its interaction with experimental treatments were considered as fixed effects (y ~ warming × precipitation level × clipping × year + (1 | block); or y ~ warming × precipitation level × clipping + year (1 | Block)). However, the corresponding model with sampling year as random intercept effect was better, based on lower Akaike information criterion (AIC) values. Thus, we decided to use the model with experimental treatments as fixed effects, with year and block as random intercept effects. Effect sizes of treatments or treatment interactions were represented by the regression coefficients in the LMMs. Wald type II χ^2 tests were used to calculate the P values from the LMMs using the 'car' R package[64]. Since the precipitation level is considered as a continuous variable in the LMM (0.5 for half precipitation, 1 for normal and 2 for double precipitation), only one regression coefficient of the precipitation treatment would be derived by the LMM. The effect size of half precipitation (as compared to ambient precipitation) can be derived by multiplying the regression coefficient by −0.5, while the effect size of double precipitation (as compared to ambient precipitation) can be derived by multiplying the regression coefficient by 1. For instance, as shown in Extended Data Fig. 2b, precipitation level was positively correlated to soil moisture with $\beta = 0.7$ per fold change (+100%) of precipitation ($P < 0.0001$; Extended Data Fig. 2b). That is, the double precipitation treatment only caused a 0.7% × 1 = 0.7% (absolute) increase in soil moisture, while half precipitation changed soil moisture by 0.7% × (−0.5) = −0.35%, that is, half precipitation decreased soil moisture by 0.35% on average.

Predicting microbial diversity with environmental variables. To link the environmental variables to microbial diversity, the correlations between the individual environmental variable and bacterial or fungal diversity were tested by the linear mixed-effects model, in which sampling year and block were termed as random intercept effects. Since richness is highly correlated with other diversity indexes (Supplementary Fig. 1), it is used to represent microbial biodiversity. The marginal coefficient of determination (pseudo-R^2) was calculated using the function 'r.squaredGLMM' of the MuMIn R package[65] to represent the variance explained by the fixed effect in the linear mixed-effects model.

Because most soil and plant predictor variables were strongly correlated with each other (Fig. 3a), we further performed a model selection analysis to compile sets of variables that are strong predictors of the data, least correlated, or potentially biologically informative based on a priori assumptions. The following tests were performed. First, the contribution of each variable on predicting bacterial or fungal richness was ranked using the bootstrap forest partitioning method, conducted by the function 'Predictor Screening' in JMP 15.0 (SAS Institute) (Supplementary Tables 3 and 4). This method evaluates the relative contribution of predictors on the response[66], and can identify predictors that might be weak alone, but strong when used in combination with other predictors. Second, the Pearson correlation coefficient between variables were calculated (Supplementary Table 5) to reveal collinearity between variables. We then applied different model selection strategies to obtain sets of variables for our linear mixed models, in which the random intercept effects of sampling year and block were included. Three alternative strategies were pursued to select soil and plant variables to be included in candidate models, on the basis of their collinearity and/or informed hypotheses: (1) Model 1 (Supplementary Tables 6 and 7 for bacteria and fungi, respectively). For highly correlated variables that have similar biological inference, we kept the one with the highest contribution on the basis of Predictor Screening. For example, we kept soil annual mean moisture rather than soil moisture at the sampling month for both LMMs on bacterial and fungal richness. For variables on plant biomass, we kept C_3 plant biomass for the LMM on bacterial richness, while we kept C_4 plant biomass for that on fungal richness. Then we iteratively removed variables with the highest correlation coefficients and kept variables that had high contributions in Predictor Screening, until the correlation between the remaining variables were less than 0.5. In this way, each set of six variables were selected for the bacterial and fungal model. (2) Model 2 (Supplementary Tables 6 and 7). We kept six least-correlated variables by removing all variables with a correlation of $r > 0.5$ using R 'caret' package[67]. (3) Model 3 (Supplementary Tables 6 and 7). The six variables with the largest contributions from Predictor Screening were kept.

The random intercept effects of sampling year and block were included in Models 1–3, which were compared on the basis of their AIC values, and the model with the lowest AIC chosen as the preferred model. Soil temperature, moisture, pH and total plant richness were selected for both bacteria and fungi, although slight differences were observed with respect to nitrogen and plant biomass (Supplementary Tables 6 and 7). These variables in the preferred model were used in the downstream structural equation modelling analyses.

Structural equation modelling. To further discern the direct and indirect effects of the environmental drivers on microbial biodiversity, SEM analyses were performed to examine the relationships among experimental treatments, soil and plant variables, and microbial diversity. To correct for potential temporal autocorrelation, we used data at the plot level, by averaging the microbial or environmental data across timepoints of the same plot. We first considered a hypothesized conceptual model (Extended Data Fig. 8) that included all reasonable pathways. Then, we sequentially eliminated non-significant pathways unless the pathways were biologically informative, or added pathways on the basis of the residual correlations. The procedure was repeated until the model showed sufficient fitting, with P values of χ^2 test >0.05 (that is, the predicted model and observed data are not significantly different) and root mean square error of approximation (RMSE) <0.08. The SEM-related analysis was performed using the lavaan R package[68].

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The DNA sequences of the 16S rRNA gene, 18S rRNA gene and ITS amplicons are deposited in the National Center for Biotechnology Information (NCBI) under project accession number PRJNA331185. Raw shotgun metagenomic sequences are deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under study no. PRJNA533082. Silva 138.1 Ref NR database is available at https://www.arb-silva.de/documentation/release-138/. Protist Ribosomal Reference database (PR2) databases are available at https://github.com/pr2database/pr2database. The ASV table and ASV representative sequences, soil physical and chemical attributes, and plant biomass and richness are downloadable online at http://www.ou.edu/ieg/publications/datasets. Source data are provided with this paper.

Code availability

R scripts for statistical analyses are available on GitHub at https://github.com/Linwei-Wu/warming_soil_biodiversity.

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Author contributions

All authors contributed intellectual input and assistance to this study. The original concepts were conceived by J.Z. and J.M.T. Field management was carried out by Linwei Wu, Y.Z., X.G., J.F., M.M.Y., J.K., Y.F., A.Z., D.N., J.M., S.J., S.H., Z.Y., Y.O. and Liyou Wu. Sampling collection, soil chemical and microbial characterization were carried out by M.M.Y., X.G., Linwei Wu, J.G., Z.G. and X.Z. Data analysis were done by Linwei Wu, Y.Z., X.G., S.L. and N.X. with assistance provided by D.N. and J.Z. All data analysis and integration were guided by J.Z. The manuscript was prepared by J.Z., Linwei Wu, Y.Z. and X.G., with significant input from J.M.T., Y.Y. and X.L. Considering their contributions in terms of site management, data collection, analyses and/or integration, Linwei Wu, Y. Z. and X.G. are listed as co-first authors.

Competing interests

The authors declare no competing interests.

Additional information

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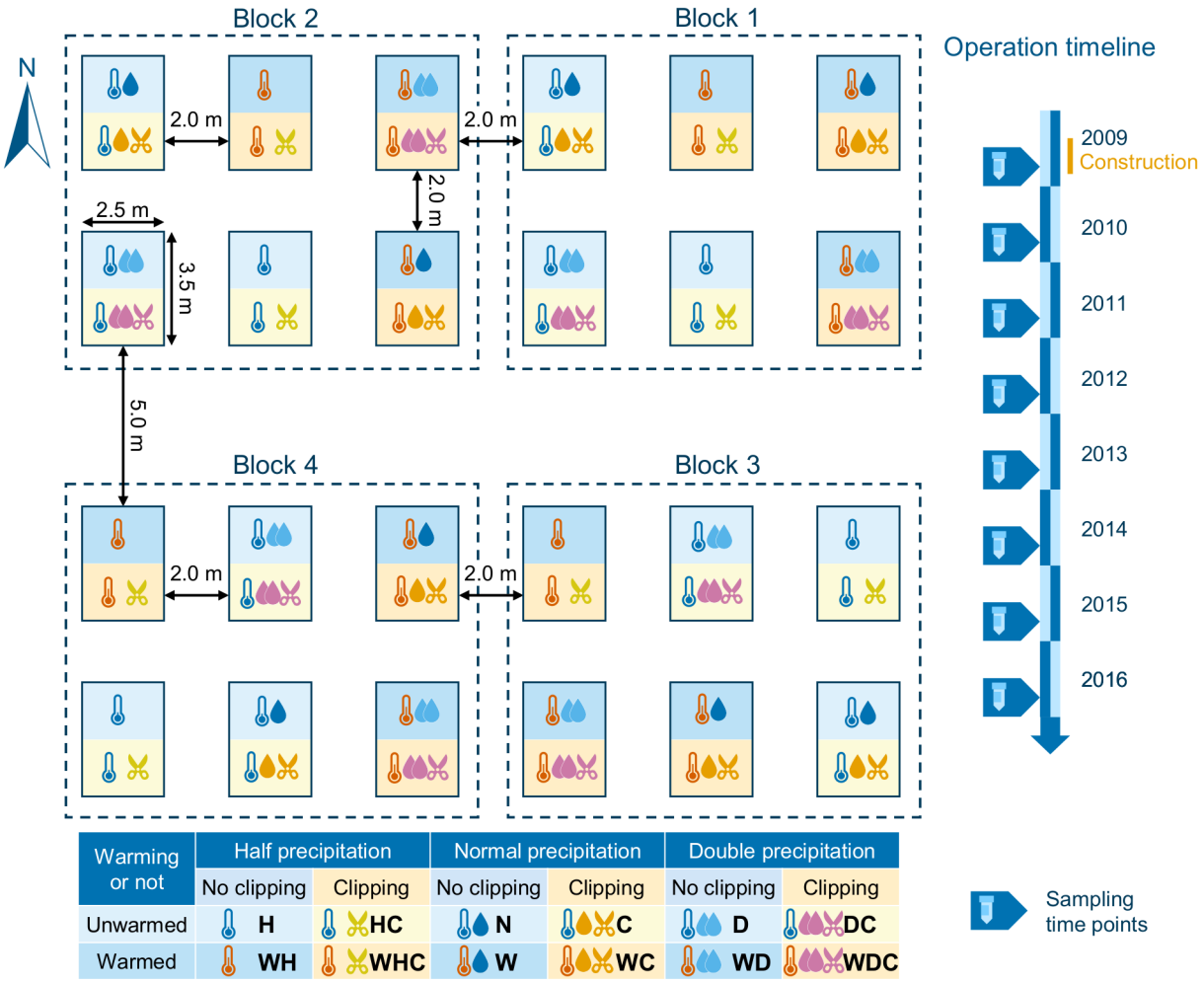
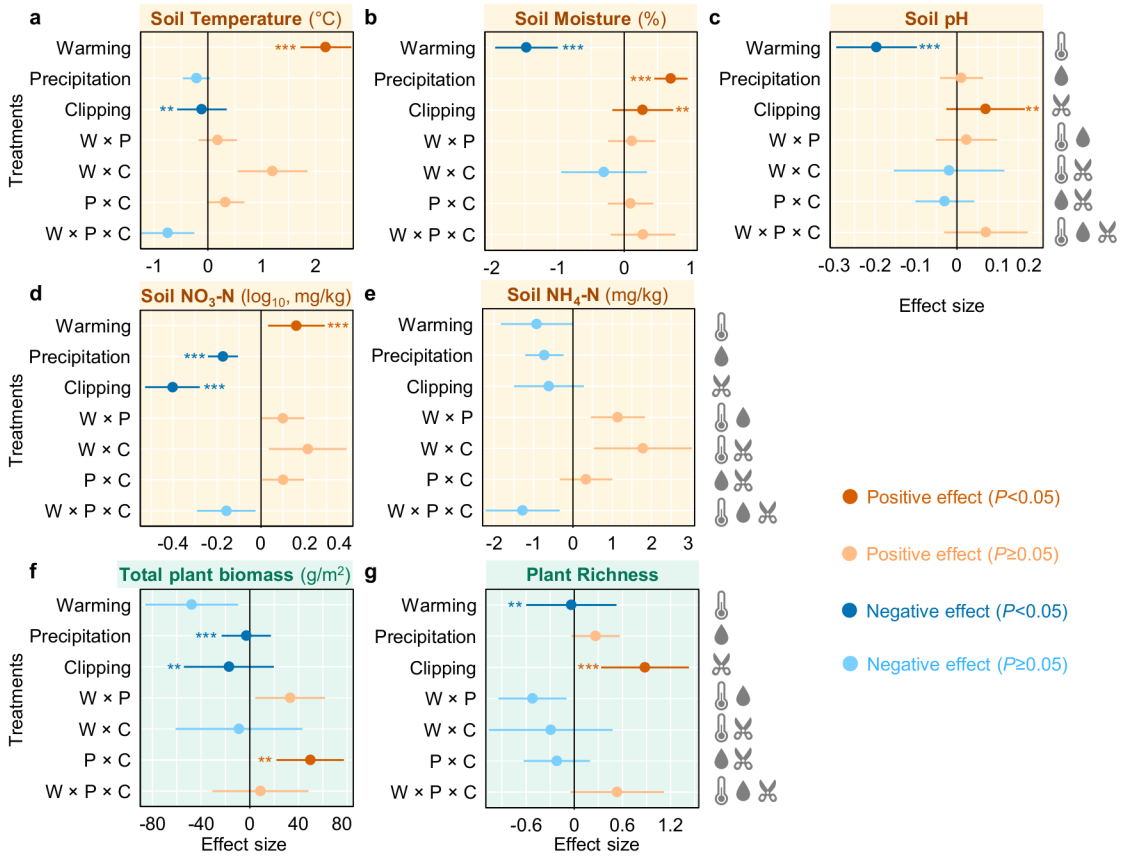
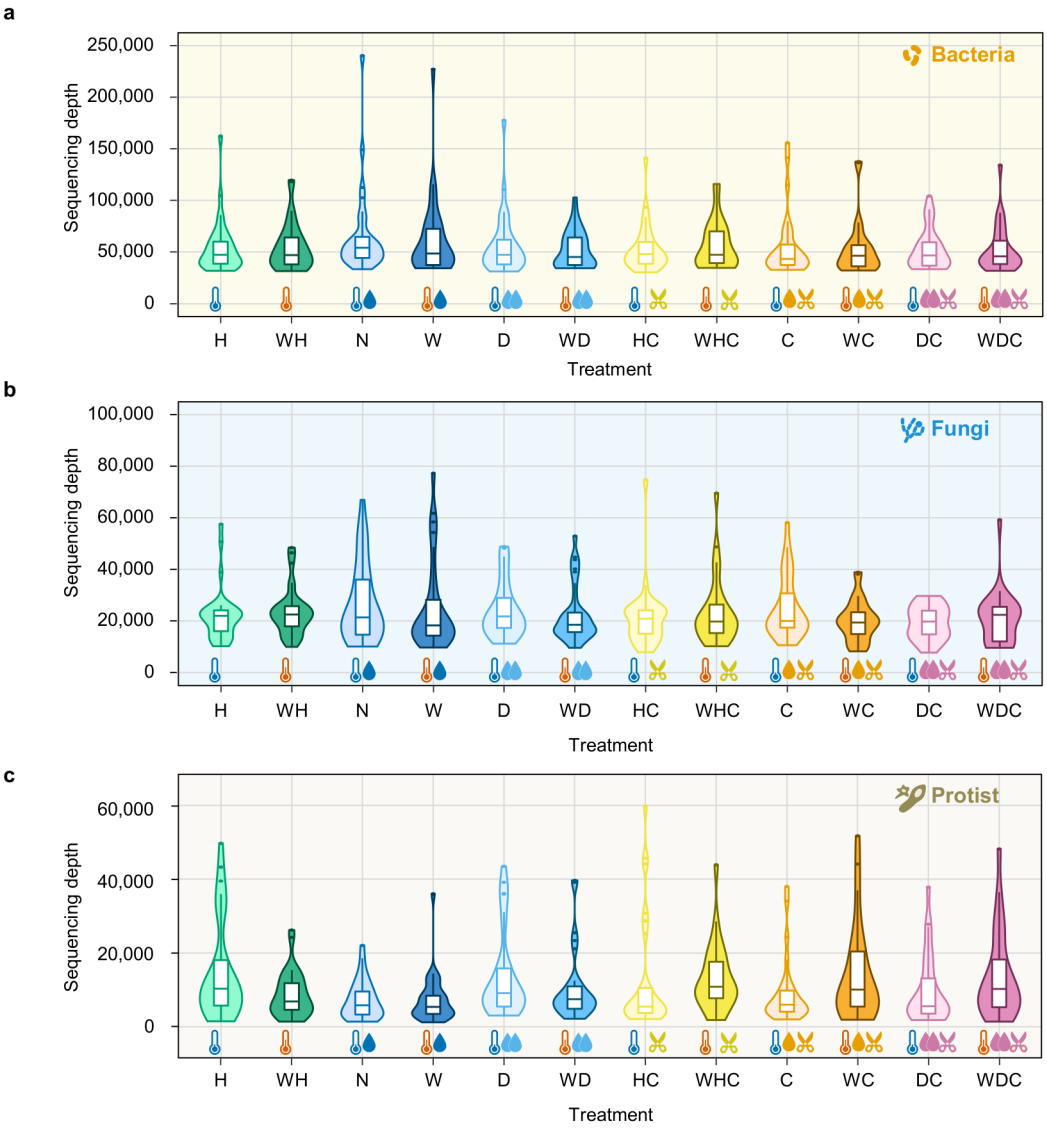


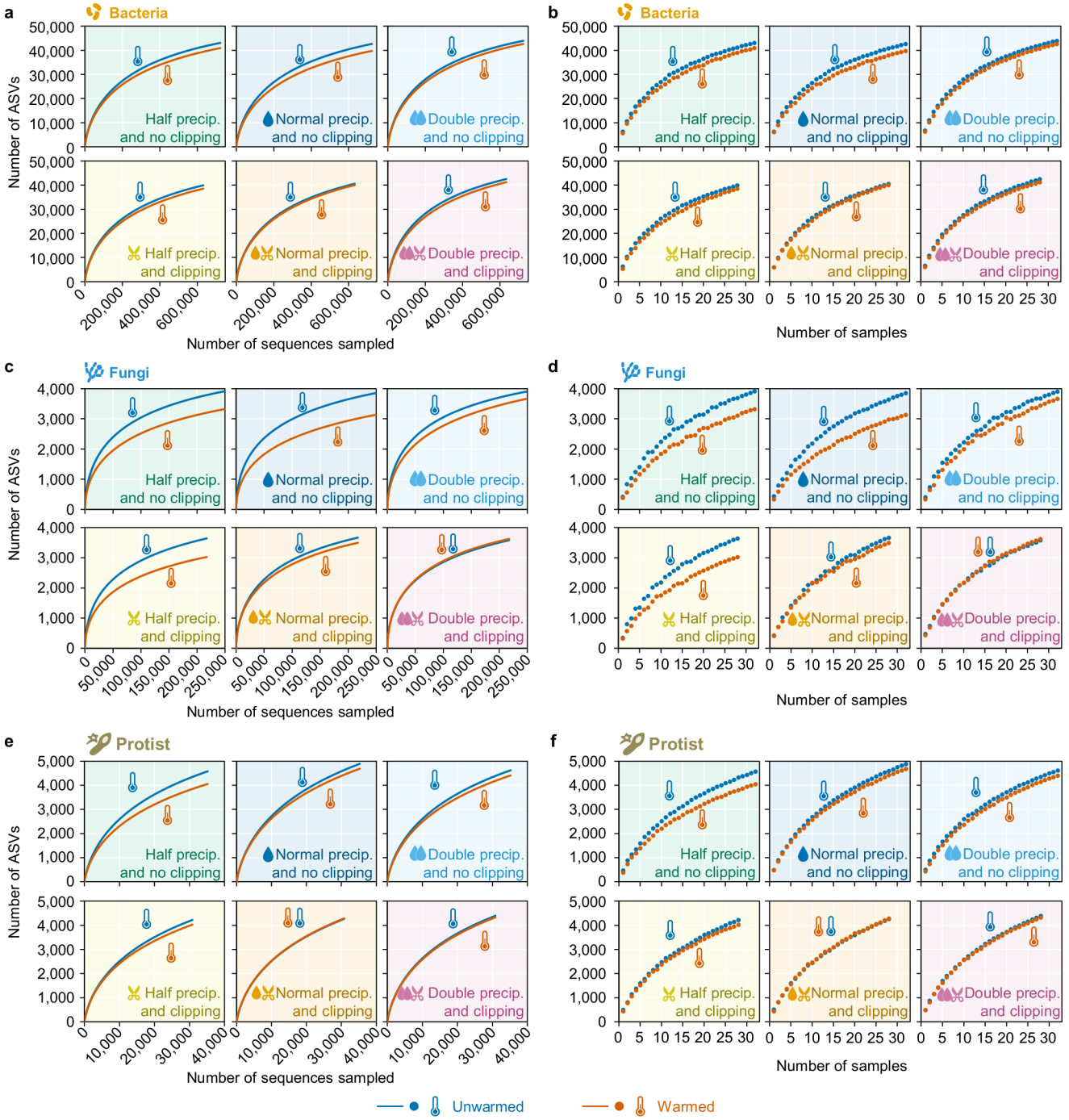
Extended Data Fig. 1 | A schematic map of the field experimental treatments. In the long-term climate change experiment, warming (+3 °C), half precipitation (−50% precipitation), and double precipitation (+100% precipitation) are primary factors, which are nested with clipping as the secondary factor (all the 24 southern subplots are under clipping treatment). Thus, this experiment has twelve single and combined treatments as follows: Control (N), Warming (W), Half precipitation (H), Double precipitation (D), Clipping (C), Warming & Half precipitation (WH), Warming & Double precipitation (WD), Warming & Clipping (WC), Half precipitation & Clipping (HC), Double precipitation & Clipping (DC), Warming & Half precipitation & Clipping (WHC), and Warming & Double precipitation & Clipping (WDC). Each of these treatments has four replicates in four different blocks. The site was established in July, 2009. Surface (0-15 cm) soil samples were collected annually from all plots at approximately the date of peak plant biomass in fall (September or October) from 2009 to 2016.



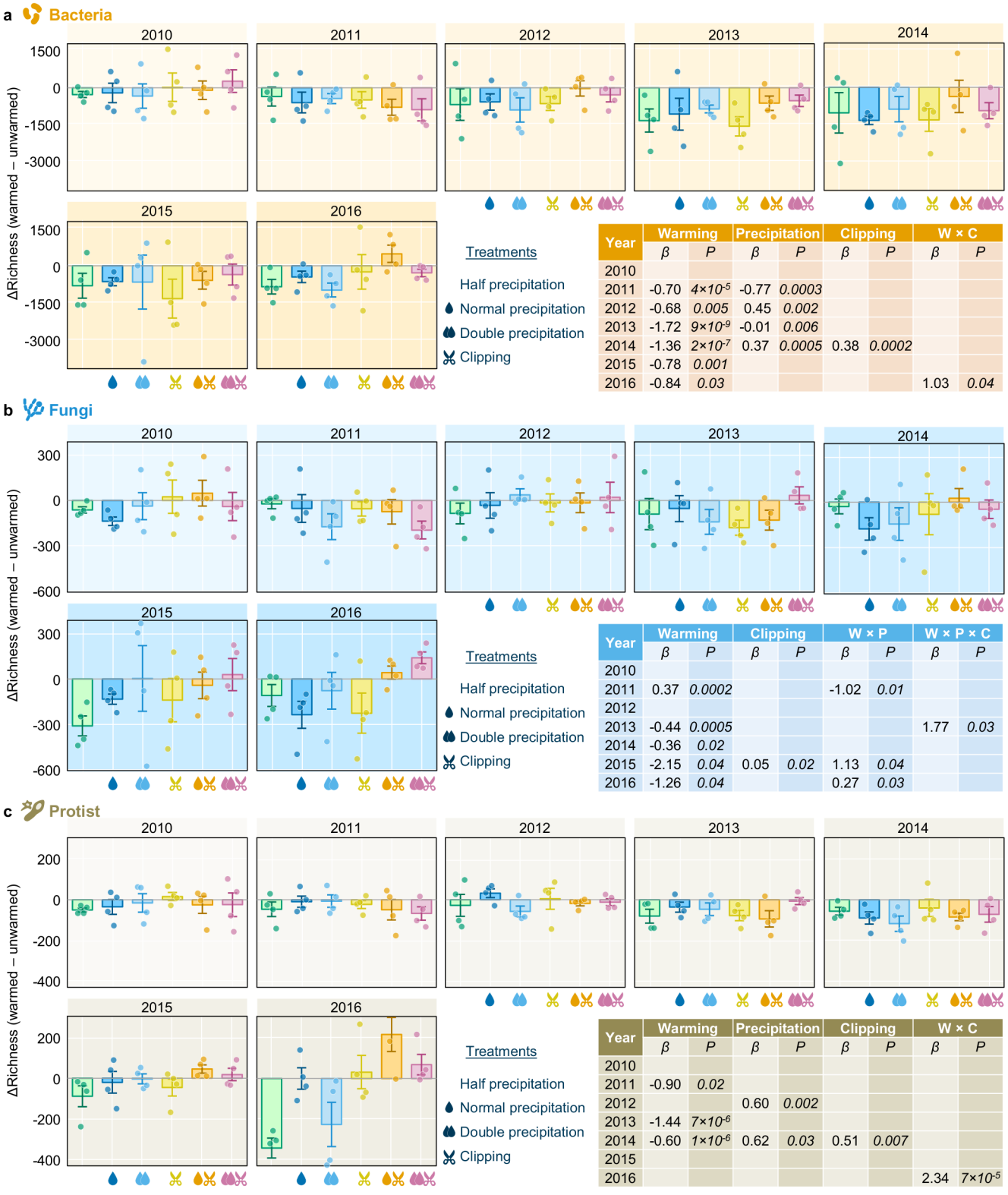
Extended Data Fig. 2 | Effects of experimental treatments on soil and plant variables by linear mixed-effects models (LMMs). a, Soil temperature; **b**, Soil moisture; **c**, Soil pH; **d**, Soil NO_3^-N; **e**, Soil NH_4-N; **f**, Total plant biomass; and **g**, Plant richness. Data are presented as mean values ± standard errors of the estimated effect sizes. Statistical significance is based on Wald type II χ^2 tests (n = 360 independent soil samples). Significant effects are denoted by asterisks: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. W: Warming; P: Precipitation level; C: Clipping. Since the precipitation level is considered as a continuous variable in the LMM (0.5 for half precipitation, 1 for normal and 2 for double precipitation), only one regression coefficient of precipitation treatment would be derived by the LMM. The effect size of half precipitation (as compared to ambient precipitation) can be derived by multiplying the regression coefficient by −0.5, while the effect size of double precipitation (as compared to ambient precipitation) can be derived by multiplying the regression coefficient by 1.



Extended Data Fig. 3 | Sequencing depth among different treatments for the bacterial community (a), fungal community (b), and protistan community (c). A total of 360 soil samples over 8 years were analyzed with 16S ribosomal RNA (rRNA) gene for bacteria and archaea, the internal transcribed spacer (ITS) between 5.8S and 28S rRNA genes for fungi, and the 18S rRNA gene for protists. For 18S sequences, only those annotated as protists were selected for the subsequent analyses. An average of 56,182 ± 27,613, 23,569 ± 16,323, and 11,146 ± 10,528 sequence reads were obtained for bacteria, fungi, and protists, respectively. There was no significant difference between treatments in the number of sequences (that is, sequencing depth) for the bacteria, fungal, and protistan communities except half precipitation (H), double precipitation (D), clipping (C), and double precipitation & clipping (DC) for protists ($p = 0.002$-0.021). Groups: Control (N), Warming (W), Half precipitation (H), Double precipitation (D), Clipping (C), Warming & Half precipitation (WH), Warming & Double precipitation (WD), Warming & Clipping (WC), Half precipitation & Clipping (HC), Double precipitation & Clipping (DC), Warming & Half precipitation & Clipping (WHC), and Warming & Double precipitation & Clipping (WDC). In the box plots, hinges show the 25, 50, and 75 percentiles. The upper whisker extends to the largest value no further than 1.5 * IQR from the upper hinge, where IQR is the inter-quartile range between the 25% and 75% quartiles; the lower whisker extends to the smallest value at most 1.5 * IQR from the lower hinge.



Extended Data Fig. 4 | Rarefaction curves. The number of ASVs with an increasing number of sequences (**a**, **c, e**) and accumulation curves of the number of ASVs with an increasing number of samples (**b**, **d, f**) for the bacterial community (**a**, **b**), fungal community (**c**, **d**), and protistan community (**e, f**). The observed number of ASVs with warming treatment was lower compared with all those without warming treatment except warming & double precipitation & clipping (WDC) versus double precipitation & clipping (DC) for fungi and warming & clipping (WC) versus clipping (C) for protists in (**a**, **c, e**) (Paired t test, $p < 0.0001$). The number of samples did not have a substantial influence on the differences between warming and non-warming control as shown in (**b**, **d, f**). After removing global singletons and resampling, the rarefaction curves approached asymptotes for all treatments, indicating that the sequencing depth was sufficient for assessing the effects of various climate change factors on the diversity of these soil microbial communities.



a Bacteria

Year	Warming		Precipitation		Clipping		W × C	
	β	P	β	P	β	P	β	P
2010								
2011	-0.70	4×10^{-5}	-0.77	0.0003				
2012	-0.68	0.005	0.45	0.002				
2013	-1.72	9×10^{-9}	-0.01	0.006				
2014	-1.36	2×10^{-7}	0.37	0.0005	0.38	0.0002		
2015	-0.78	0.001						
2016	-0.84	0.03					1.03	0.04

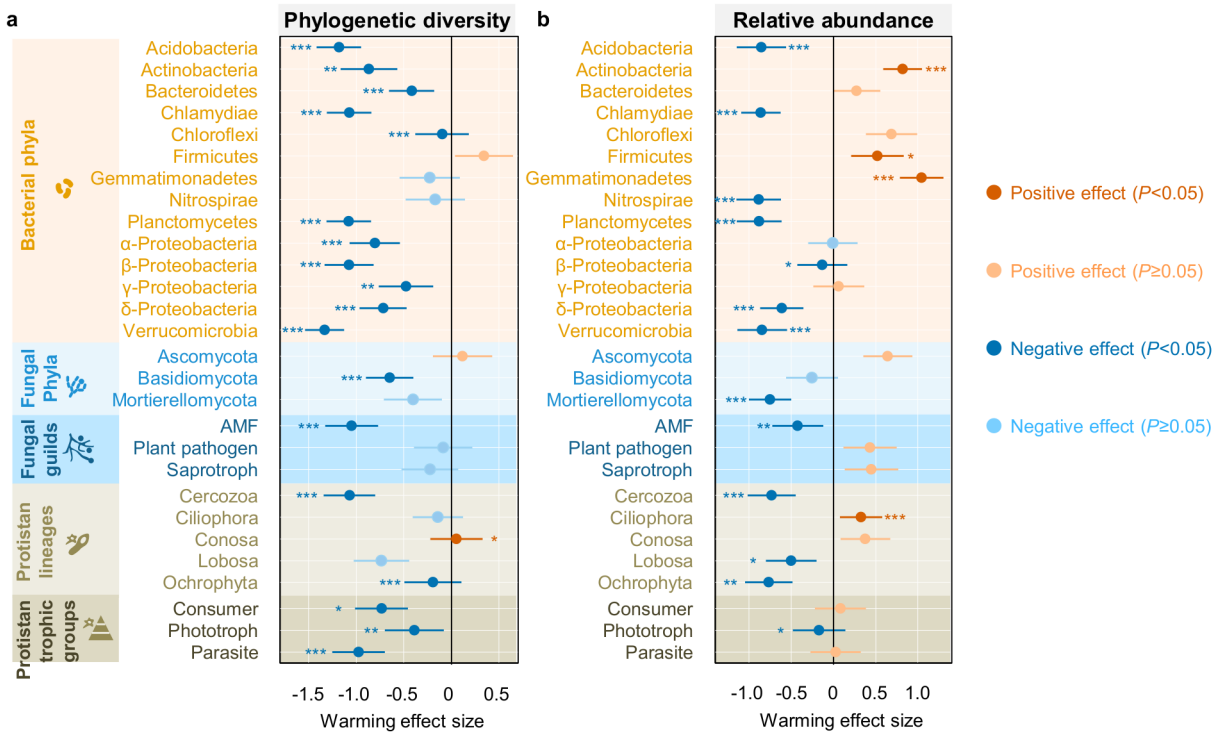
b Fungi

Year	Warming		Clipping		W × P		W × P × C	
	β	P	β	P	β	P	β	P
2010								
2011	0.37	0.0002			-1.02	0.01		
2012								
2013	-0.44	0.0005					1.77	0.03
2014	-0.36	0.02						
2015	-2.15	0.04	0.05	0.02	1.13	0.04		
2016	-1.26	0.04			0.27	0.03		

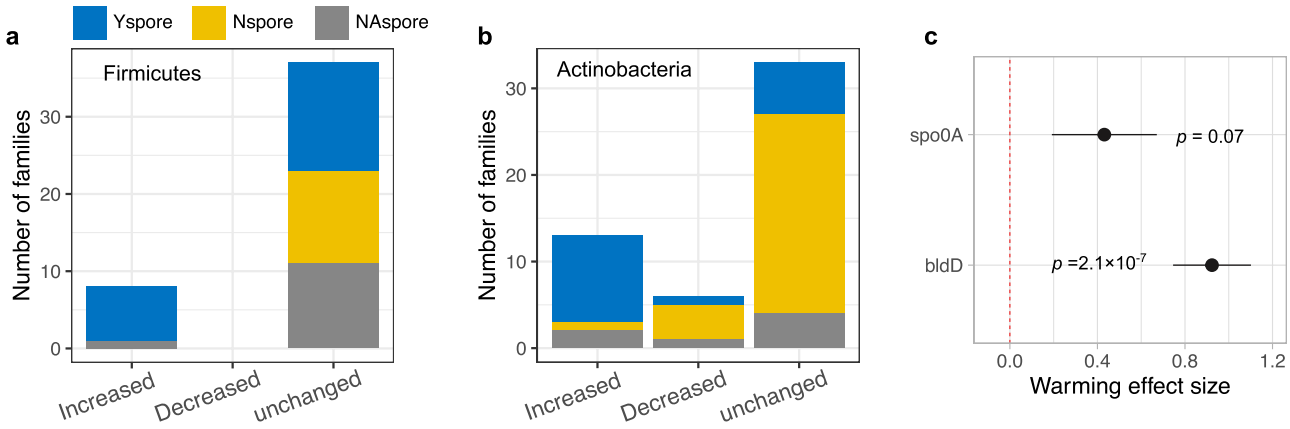
c Protist

Year	Warming		Precipitation		Clipping		W × C	
	β	P	β	P	β	P	β	P
2010								
2011	-0.90	0.02						
2012			0.60	0.002				
2013	-1.44	7×10^{-6}						
2014	-0.60	1×10^{-6}	0.62	0.03	0.51	0.007		
2015								
2016							2.34	7×10^{-5}

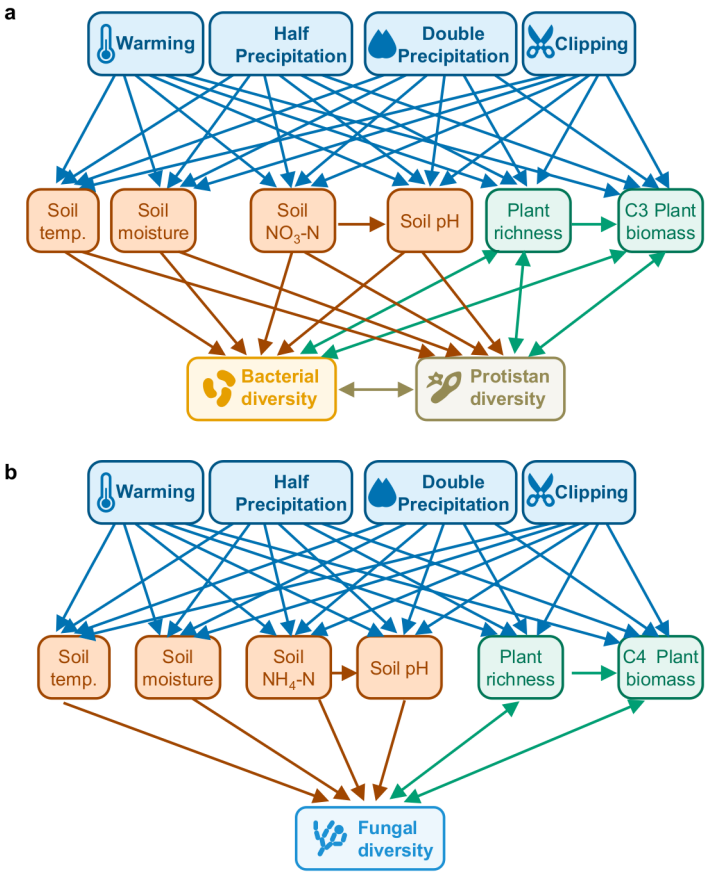
Extended Data Fig. 5 | Yearly differences of bacterial (a), fungal (b), and protistan (c) richness between warmed and unwarmed samples. Data are presented as mean values ± SEM of the mean differences (warmed -unwarmed). For each year, the treatment effects are tested with linear mixed-effects models, and the significant treatment effects ($p < 0.05$, Wald type II χ^2 tests, $n = 48$ soils each year) are listed in the table. W: Warming; P: Precipitation level; C: Clipping.



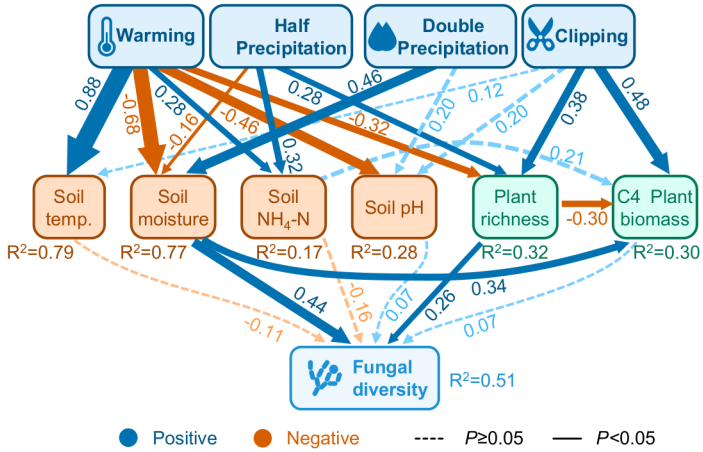
Extended Data Fig. 6 | Effects of experimental warming across microbial groups based on linear mixed-effects models. a, Effect sizes of experimental warming on the (rescaled) phylogenetic diversity of major microbial groups based on linear mixed-effects models. **b**, Effect sizes of experimental warming on the (rescaled) relative abundance of major microbial groups based on linear mixed-effects models. Data are presented as mean values ± standard errors of the estimated effect sizes. Statistical significance is based on Wald type II χ^2 tests (n = 360 independent soil samples), which is denoted by asterisks: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.



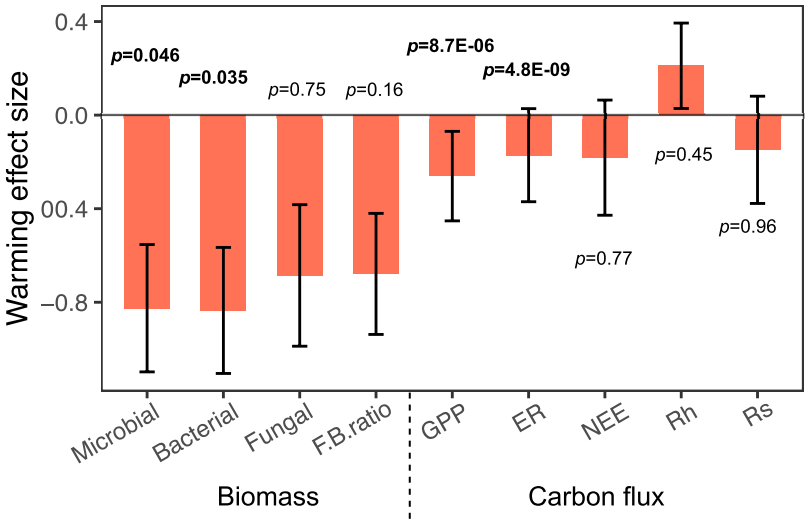
Extended Data Fig. 7 | Effects of experimental warming on sporulation families or genes of Firmicutes and Actinobacteria. a, Number of Firmicutes families whose relative abundances increased, decreased or unchanged under warming. **b**, Number of Actinobacteria families whose relative abundances increased, decreased or unchanged under warming. Significant changes ($p < 0.05$) are based on Wald type II χ^2 tests ($n = 360$ independent soil samples) of the warming effects in linear mixed-effects models (relative abundance ~ warming × precipitation level × clipping + (1|Block) + (1|year)). 'Yspore', 'Nspore', and 'NAspore' refer to known spore-formers, known non-spore-formers, and information not available on the ability to form spores, respectively. The spore-forming capability or different families within Firmicutes and Actinobacteria were mainly based on databases and literature. **c**, Effect sizes of experimental warming on the (rescaled) relative abundance of major sporulation genes in Firmicutes (spo0A gene) and Actinobacteria (bldD gene) based on linear mixed-effects models. The sporulation genes were retrieved from shotgun sequencing data. Data are presented as mean values ± standard errors of the estimated effect sizes. Statistical significance is based on Wald type II χ^2 tests ($n = 64$ independent soil metagenome samples).



Extended Data Fig. 8 | Hypothesized conceptual models on the relationships between experimental treatments, environmental variables, and microbial diversity. a, Bacteria and protists; **b**, Fungi. The environmental variables were selected based on their biological inference and collinearity, as detailed in the Methods and Supplementary Tables 3–7. We hypothesized that each experimental treatment would influence each environmental variable, and the environmental variables would all influence microbial diversity. We also assumed that microbial diversity would influence plant richness and biomass. In addition, we assumed interactions between bacteria and protists since there could be prey-predator relationships between them. In fact, the consumers, which include potential predators of bacteria, account for 84.6% of the total protist abundance. The richness of protistan consumers also highly correlated with the total protistan richness (Pearson's $r = 0.98$). Protists was not included in the fungal model because the relative abundance of fungivorous protists is very low.



Extended Data Fig. 9 | The structural equation model (SEM) showing the relationships among treatments, soil and plant variables, and fungal richness. Blue and red arrows indicate positive and negative relationships, respectively. Solid or dashed lines indicate significant ($p < 0.05$) or nonsignificant relationships. Numbers near the pathway arrow indicate the standard path coefficients. R^2 represents the proportion of variance explained for every dependent variable. $\chi^2 = 28.70$, df = 23, $p = 0.19$ (large p value indicates that the predicted model and observed data are equal, that is, good model fitting), CFI = 0.974. n = 48 biologically independent plots.



Extended Data Fig. 10 | Effects of experimental warming on different ecosystem functions. Data are presented as mean values ± standard errors of the estimated effect sizes. Statistical significance is based on Wald type II χ^2 tests (n = 360), which is indicated in the plot. GPP: gross primary productivity; ER: ecosystem respiration; NEE: net ecosystem exchange; Rh: heterotrophic respiration; Rs: soil total respiration.

nature portfolio

Corresponding author(s): Jizhong Zhou

Last updated by author(s): Apr 30, 2022

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used to collect the data in this study.
Data analysis	Amplicon sequencing data processing was done using the Galaxy Server (usegalaxy.org)-based pipeline published and developed by the Institute for Environmental Genomics, University of Oklahoma accessible by public at http://zhoulab5.rccc.ou.edu:8080/root. The paired-end sequences were merged using FLASH (http://www.cbcb.umd.edu/software/flash). The high-quality 16S rRNA gene, ITS or 18S rRNA gene sequences were processed to generate amplicon sequence variants (ASVs) by UNOISE3 (https://www.drive5.com/usearch/manual/tutorials.html). The representative 16S rRNA or 18 rRNA gene sequences were aligned using Clustal Omega v1.2.2 for constructing the phylogenetic tree by FastTree2 v2.1.10. For 16S rRNA and ITS genes, the ASVs or OTUs were taxonomically annotated with RDP Classifier (http://rdp.cme.msu.edu/classifier/classifier.jsp); ITS sequences were further assigned into functional groups using FUNGuild (https://github.com/UMNFuN/FUNGuild). The quality of the metagenomic data was evaluated using FastQC v0.11.6. CD-HIT was used to remove duplicates with an identity cutoff of 100%. NGS QC Toolkit (version 2.3.3) was used for quality filtering. High-quality reads were searched against NR database (BLASTx) using DIAMOND (https://github.com/bbuchfink/diamond). The outputs were submitted to MEGAN6 (Ultimate Edition, version 6.6) for function profiling. Statistical analyses were done using R software 4.0.0. R scripts are available on GitHub at https://github.com/Linwei-Wu/warming_soil_biodiversity.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The DNA sequences of the 16S rRNA gene, 18S rRNA gene and ITS amplicons were deposited to the National Center for Biotechnology Information (NCBI) under the project accession number PRJNA331185.
Raw shotgun metagenomic sequences are deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under study no. PRJNA533082.
Silva 138.1 Ref NR database is available at https://www.arb-silva.de/documentation/release-138/.
Protist Ribosomal Reference database (PR2) databases is available at https://github.com/pr2database/pr2database.
The ASV table and ASV representative sequences, soil physical and chemical attributes, and plant biomass and richness are downloadable online at http://www.ou.edu/ieg/publications/datasets.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	The field experiment was conducted at the Kessler Atmospheric and Ecological Field Station (KAEFS) of the US Great Plain in McClain County, Oklahoma, USA (34̊ 59′ N, 97̊ 31′W). This experiment used a blocked split-plot design, in which warming (continuous heating at a target of +3 °C above ambient) and precipitation alteration (target of -50% and +100%) were primary factors nested with annual removal of aboveground biomass in peak growth season as the secondary factor. Thus, this experiment has 2*3*2=12 different treatment groups. Each of these treatments has 4 replicates in different four blocks, that is, a total of 48 subplots.
Research sample	Before the experiment started (i.e., year 2009), 24 pre-warmed surface (0 - 15 cm) soil samples were collected using a soil core as the background of comparison. For the following years (i.e., from year 2010 to 2016), a total of 336 samples were collected during the experiment, one sample per plot per year. With the blocked split-plot design of the experimental site, annual soil samples were taken in peak growth season from warming (continuous heating at a target of +3 °C above ambient) and precipitation alteration (target of -50% and +100%) nested with annual removal of aboveground biomass as the secondary factor (4 replicates for each treatment). Soil microbial DNA was extracted from 1.5 g of well-mixed soil for each sample in order to investigate the richness and abundance distribution of microbial (e.g., bacterial, fungal, and protistan) communities over the years.
Sampling strategy	Sampling strategy was determined together with the site design. With the 2*3*2=12 different treatment groups of the study site (see details in Study description), 4 biological replicates located in each of the 4 blocks for each treatment were sampled annually (from 2010 to 2016). These 336 samples from 12 different treatment groups would be sufficient for measuring variation and evaluating differences in and between treatments using statistical tests, such as linear mixed-effects models. The surface (0 - 15cm) soil samples were taken using a standard soil corer (2.5 cm in diameter). The surface (0 - 15cm) soil samples were taken using a standard soil corer (2.5 cm in diameter). About 50 g of soil sampled were mixed well before 1.5 g of soil was weighed for DNA extraction from each plot/timepoint.
Data collection	Soil temperature was measured every 15 min at depth of 7.5, 20, 45 and 75 cm in the center of each plot using constantan-copper thermocouples wired to a Campbell Scientific CR10x data logger (Campbell Scientific). The data were recorded and backed up by Daliang Ning in computers periodically. Soil chemical properties, including total carbon (C), total nitrogen (N), nitrate (NO3-) and ammonia (NH4+), were analyzed in the Soil, Water, and Forage Analytical Laboratory at Oklahoma State University (Stillwater, OK, USA). The results were sent to Linwei Wu through emails and stored in computers. Volumetric soil water content (%V) was measured using a portable time domain reflectometer (Soil Moisture Equipment Corp.) once or twice a month, and recorded by pen and paper. Ecosystem carbon (C) fluxes were measured once or twice a month between 10:00 and 15:00 (local time). Net ecosystem exchange and ecosystem respiration were measured using an LI-6400 portable photosynthesis system (LI-COR) attached to a transparent chamber (0.5 m×0.5 m×0.7 m), which covered all of the vegetation within the aluminium frames. The LI-6400 system had storage to record the data. Soil total respiration and heterotrophic respiration were measured using a LI-8100A soil flux system attached to a soil CO2 flux chamber (LI-COR). The LI-8100A system had storage to record the data. Meanwhile, a manual record (by pen and paper) was also kept. Soil water content and ecosystem carbon fluxes were measured monthly or biweekly by the field team, including Linwei Wu, Ya Zhang, Xue Guo, Daliang Ning, Liyou Wu, Xishu Zhou, Jiajie Feng, Mengting Maggie Yuan, Jialiang Kuang, Ying Fu, and Naijia Xiao. The measurements were recorded 16S and ITS amplicon sequencing was performed following a published protocol (Wu 2015) on Illumina MiSeq platform.
Timing and spatial scale	The experimental site was initiated in July of 2009. Data and samples for this study were collected since then to 2016 (ending date: Dec 22, 2016). The samples/data collected in 2009 were used the background of comparison (before the experiment started) and the samples/data collected in the following years 2010-2016 were during the warming experiments. This study was to investigate the

	richness and abundance distribution of microbial (e.g., bacterial, fungal, and protistan) communities over the years. Therefore, one soil sample were collected in each plot once every year. Soil temperature was measured every 15 min at depth 7.5 cm and annual average values of temperature were used to represent soil temperature across experimental years. Volumetric soil water content (% V) was measured once or twice a month, and annual average values were used to represent soil moisture. Ecosystem carbon (C) fluxes were measured once or twice a month between 10:00 and 15:00 (local time) and annual average values were used in analyses. There were no gaps between collection periods. The site has four experimental blocks, each including six plots. Each plot has the size of 2.5×3.5 m2.
Data exclusions	No data were excluded.
Reproducibility	Our data were from 4 biological replicates located in each of the 4 blocks for each treatment (spatially). They were time series data collected annually from 2009-2016 (temporally). All the attempts at replicates were successful.
Randomization	The plots within each block were assigned to different treatment randomly.
Blinding	All the soil sample processing and field measurements were done following the same way, and without signs/labels noting the relavant treatment.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	The field experiment was conducted at the Kessler Atmospheric and Ecological Field Station (KAEFS) of the US Great Plain in McClain County, Oklahoma, USA (34 59′ N, 97 31′W). The average air temperature was 16.3 °C and the average annual precipitation was 914 mm, based on Oklahoma Climatological Survey from 1948 to 1999. The experimental site was dominated by C3 forbs (Solanum carolinense, Ambrosia trifida and Euphorbia dentate), C3 grasses (Bromus sps) and C4 grasses (Tridens flavus and Sorghum halapense). The soil type was Port-Pulaski-Keokuk complex, with a neutral pH, a high available water holding capacity (37%) and a deep (ca. 70 cm), moderately penetrable root zone.
Location	The field experiment was conducted at the Kessler Atmospheric and Ecological Field Station (KAEFS) of the US Great Plain in McClain County, Oklahoma, USA (34 59′ N, 97 31′W). See KaEFS website for more details (https://www.ou.edu/kaefs).
Access & import/export	The property on which the field experiment was built belongs to the University of Oklahoma. The acting director of the site is Meghan Bomgraars (mbomgaars@ou.edu, 405-325-5202). The authors have full access to the field site to conduct research. All the research activities conducted on site complies to national and local laws and regulations, and rules imposed by the University of Oklahoma in terms of ecological conservation and work safety.
Disturbance	The field experimental site was fenced with a buffer area without significant disturbance to the tall grass prairie around.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging