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Opposite effects of N on warming-induced changes in bacterial and fungal diversity

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Abstract

The diversity of bacteria and fungi is linked to distinct ecosystem functions, and divergent responses to global changes in these two kingdoms affect the relative contributions of the kingdoms to the soil carbon and nutrient cycles. Climate warming and nitrogen (N) enrichment, which are projected to increase concurrently through modelling efforts, are considered the main drivers of biodiversity loss. However, it is unclear how bacterial and fungal diversity respond differently to the simultaneous occurrence of climate warming and nitrogen enrichment, and the underlying mechanisms involved remain unknown. Using a 9-yr warming and N enrichment experiment in an alpine permafrost area of the Tibetan Plateau, we demonstrated the contrasting response of bacterial and fungal diversity to combined warming and N enrichment, showing a reduction in bacterial richness (8.8%) and an increase in fungal diversity (33.6%). Furthermore, the negative effects of warming on fungal richness were reversed by N enrichment, and the negative effects of nitrogen enrichment on bacteria were amplified by warming. Our results also demonstrated that both biotic interactions, such as bacterial–fungal antagonism, and abiotic factors, primarily the soil C/N ratio and pH, play crucial roles in shaping microbial biodiversity. Our findings suggest that fungal diversity is expected to greatly increase in a warmer and more nitrogen-enriched world, potentially leading to the enhancement of ecosystem functions driven by fungi.

Introduction

Soil microbial biodiversity is vital for sustaining ecosystem functions and stability [1–3]. Bacteria and fungi are the main soil microbes, and the diversity of these two kingdoms plays distinct and important roles in ecosystem functions due to their unique physiological properties [4–6]. However, global factors, including climate change and anthropogenic disturbances, may have different impacts on bacterial and fungal diversity [7, 8], changing the relative contributions of these two kingdoms to soil carbon and nutrient cycling. For example, a warming experiment conducted over 7 consecutive years in a grassland revealed a greater reduction in fungal diversity than bacterial diversity [9], while 3 years of nitrogen (N) fertilization in a semiarid grassland increased fungal diversity but decreased bacterial diversity [10]. Climate warming and nitrogen deposition are consistently predicted to increase in the future based on modelling efforts [11, 12], and recognized as the main drivers of biodiversity loss in this century [8, 13, 14]. However, how bacterial and fungal diversity respond to the combined of climate warming and nitrogen enrichment, particularly with respect to the underlying mechanisms involving environmental conditions and biotic interactions, remains unclear.

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Bacteria and fungi may have opposite responses to global changes due to their different sensitivities to shifts in environmental conditions, such as abiotic stress and nutrients [15]. Fungi are generally considered more well-suited for survival than bacteria under abiotic stress conditions involving high acidity, low moisture or high salinity; their high stress resistance may be explained by their unique hyphal structure and complex genome functional traits associated with stress tolerance [16–18]. In contrast, bacteria are more dominant than fungi when soil resources are abundant because bacteria tend to have more efficient resource acquisition capacity and lower metabolic requirements [19, 20]. Climate warming and N enrichment may lead to diverse abiotic stresses related to decreased soil moisture and soil pH [9, 21], which are associated with increased fungal diversity. Moreover, these conditions can increase soil resource quantity by promoting plant productivity and increasing organic matter inputs to soil [22], which increases bacterial diversity. However, how these environmental shifts mediate the effects of climate warming and N enrichment on bacterial and fungal diversity is unclear.

In addition to abiotic environmental factors, biotic interactions, such as interkingdom antagonism, can play a substantial role in shaping microbial diversity [20, 23, 24]. The strength of bacterial–fungal antagonism has been shown to correlate with the prevalence of fungi, which is reflected by the bacterial/fungal biomass ratio [23]. To outcompete bacteria, many fungal taxa secrete large amounts of antimicrobial compounds, which can suppress bacterial growth [4, 25]. Bacteria with antibiotic resistance genes (ARGs) are more likely to survive fungal antagonism. As a result, this antagonistic interaction screens out bacteria lacking ARGs, leading to an enrichment in ARG-carrying bacteria and a reduction in bacterial diversity [4, 23]. Although the relationship between bacterial–fungal antagonism and microbial diversity has been demonstrated at large spatial scales [23], it remains unclear in the context of global change. Warming typically reduces fungal biomass [26, 27], indicating a potential decrease in the strength of bacterial–fungal antagonism. In contrast, elevated nutrient inputs may increase bacterial–fungal antagonism due to the consistently observed increases in the relative abundance of copiotrophic bacteria [28], such as Proteobacteria, which have the greatest average number of ARGs per genome among bacteria [23]. However, little is known about whether and how fungal–bacterial antagonism shapes bacterial and fungal diversity under climate warming and nitrogen enrichment.

To determine whether and how climate warming and nitrogen enrichment affect soil bacterial and fungal diversity, soil samples were collected from a 9-year warming and N-addition field experiment on the Tibetan Plateau. We measured the taxonomic diversity of soil bacteria and fungi via high-throughput sequencing of marker genes. In addition, we investigated the strength of bacterial-fungal antagonism by analysing antibiotic-resistance gene abundance from the soil metagenomic samples. We focused on the following major questions: do bacterial and fungal diversity respond differently to experimental warming and nitrogen enrichment; and how alterations in environmental conditions and biotic interactions mediate these responses? We hypothesize that climate warming and nitrogen enrichment lead to an increase in fungal diversity and a loss of bacterial diversity due to the increasing strength of bacterial–fungal antagonism and environmental conditions that favour fungi.

Methods

Study site and sampling

This study was conducted in an alpine meadow located on the Tibetan Plateau, Maqu County, Gansu Province, China (101°53′ E, 35°58′ N, 3500 m above sea level). The region has a continental climate with a mean annual temperature of 1.2 °C and a mean annual precipitation of 620 mm.

A field-based warming and N-addition experiment was established with 48 plots base on a split-plot block design in June 2011. All plots (5 × 5 m) were separated from adjacent plot edges by 1 m buffer zones, and had roughly the same species diversity and community structure. These 48 plots were randomly selected for four treatments with different rate of nitrogen addition (in the form of NH_4NO_3, which is the main form of exogenous N on the Tibetan Plateau): 0, 5, 10, and 15 g m^{-2} yr^{-1}. Half of each nitrogen treatment plot was also randomly selected for warming via transparent open-top 1.5-m^2 chambers (OTCs). Specifically, there were eight treatments, comprising N enrichment (N0, N1, N2, and N3) and warming combined with N enrichment (WN0, WN1, WN2, and WN3), with six replicates per treatment. The low nitrogen dose represented the observed N enrichment due to grazing (up to ~ 6.0 g N m^{-2} yr^{-1}) and N deposition (0.1–6.4 g N m^{-2} yr^{-1}) in this region, and the high dose allowed us to gain insights into the effects of N enrichment on ecosystem processes under current and future scenarios. For the warming treatment, the soil temperature at a depth of 10 cm increased by 1.03 °C. Soil cores from the surface layer (0–10 cm) were collected from each treatment plot by using a 4 cm diameter auger in August 2019. The fresh soil samples were then immediately transported to the laboratory on ice. In the laboratory, the samples were homogenized, all visible stones and plant roots in the soil were removed, and the soil was

passed through a 2 mm sieve before DNA extraction and soil chemical measurements.

Soil chemical measurements

Soil pH was determined using a pH meter (PHS-3D, Rex, Shanghai, China) in a 1:5 soil-to-water suspension. For SOC and TN analysis, air-dried soil was ground and fumigated with HCl, after which the SOC and TN content was determined using an elemental analyser (FlashSmart, Thermo Fisher Scientific, USA). Soil DOC concentrations in both nonfumigated and fumigated soils were measured with a TOC analyser (Multi N/C 3100, Analytik Jena GmbH, Germany).

DNA extraction and sequencing

For each soil sample, microbial DNA was extracted from 1.5 g of soil using a DNeasy PowerSoil Pro Kit (Qiagen) following the manufacturer's protocol. DNA quality and concentration were evaluated on the base of 260/280 nm absorbance ratios using a NanoDrop spectrophotometer. DNA samples were stored at − 80 °C until use. To study the diversity of the soil microbial community, we targeted the V3-V4 hypervariable region of the 16S rRNA gene for bacteria, and the first internal transcribed spacer (ITS1) region of the rRNA operon for fungi. The target sequences were amplified by PCR with the primer pairs 338F/806R (338F: 5′-ACT CCT ACG GGA GGC AGC A-3′; 806R: 5′-GGA CTA CHV GGG TWT CTA AT-3′) for bacteria and ITS1F/ITS2 (ITS1F: 5′-CTT GGT CAT TTA GAG GAA GTA A-3′; ITS2: 5′-GCT GCG TTC TTC ATC GAT GC-3′) for fungi. The amplicon libraries were sequenced on an Illumina HiSeq 2500 platform with 250 bp paired-end reads. The metagenome libraries were constructed from genomic DNA and sequenced via the Illumina NovaSeq 6000 platform.

Metabarcoding analyses

The paired-end sequences were merged via USEARCH [29] with the `fastq_mergepairs` command, followed by primer sequence trimming with the `search_prc2` command. Merged sequences shorter than 150 bp were discarded. Lower-quality sequences were further removed via the USEARCH `fastq_filter` command (maxee < 1). An average of 50,749 ± 959 and 53,750 ± 4926 sequence reads were obtained for the 16S rRNA gene and ITS, respectively. These high-quality 16S rRNA gene and ITS sequences were clustered to generate amplicon sequence variants (ASVs; also known as unique sequence variants and zero-radius operational taxonomic units (ZOTUs)) at a 100% similarity threshold by UNOISE3 [30]. The raw sequences were then mapped against these amplicon sequence variants to generate an ASV counts table using the `otutab` command in USEARCH. Taxonomy was assigned to each ASV via the RDP classifier using 16S rRNA gene training set 16 [31] for bacteria and the UNITE Fungal ITS training set (version of August 2022) [32] for fungi. For 16S rRNA, ASVs classified as chloroplast and mitochondria were removed before further processing. To normalize the sequencing depth across samples, samples were rarefied to 30,689 bacterial, and 34,451 fungal sequences per sample. The 16S rRNA gene copy number of each ASV was estimated by annotation against the rrnDB database [33]. Bacterial species were classified as oligotrophs if they had 1 or 2 rrn copies or as copiotrophs if they had 3 or more rrn copies [34]. To divide fungi into functional groups—plant pathogens, ectomycorrhizal fungi (EMFs), arbuscular mycorrhizal fungi (AMFs) and saprotrophs, ITS sequences were further assigned via FUNGuild [35].

ARG gene profiling from the metagenomic dataset

The raw metagenomic reads were quality-filtered via Trimmomatic, retaining those with an average quality score ≥ 20. High-quality reads from the six replicates of each treatment were pooled and coassembled via MEGAHIT v.0.39 [36], with a minimum contig length of 1000 bp (–k-min 27 –k-max 127 –k-step 20 –min-contig-len 1000). Gene prediction was performed on the assembled contigs using Prodigal (v2.6.3) [37]. The gene counts for each individual sample were obtained by mapping the raw sequences back to the predicted genes via BWA v.2.2.1 [38] with default parameters. To identify ARGs in our metagenome samples, the translated proteins from all the detected genes were mapped to CARD [39] reference Databases v.3.2.4 by their recommended tool, RGI v.6.0.3. with the parameters "-t protein -a diamond". The relative abundance of ARGs was calculated by normalizing the total counts of ARG genes to the total number of detected genes in each sample.

Statistical analysis

Richness was used to measure taxonomic α-diversity and was calculated as the total number of observed ASVs. Other taxonomic α-diversity indices, including the Shannon index and Chao1 index, were calculated using the 'vegan' R package. Significant differences in richness among the different treatments were examined via two-way ANOVA followed by Tukey's HSD post hoc test ($P < 0.05$ was considered statistically significant). Richness at the phylum level or within functional groups was calculated by categorizing ASVs on the basis of their taxonomic classifications or functional guild annotations, respectively, and summing the total number of ASVs observed within each phylum or functional group. A summary of the number of sequences identified for each phylum and functional

group is provided in Table S1. Significant differences in microbial group richness between the treatment (W, N1, N2, N3, WN1, WN2, WN3) and control (N0) were examined by two-tailed t test. The relationships between the relative abundances of ARGs and microbial diversity were evaluated via general linear regression. All the statistical analyses were conducted via R version 4.2.1.

To evaluate the direct and indirect effects of the environmental drivers on bacterial and fungal diversity, structural equation modelling (SEM) was performed to examine the relationships among the experimental treatments, soil variables and microbial diversity. We initially proposed a hypothesized conceptual model that included all reasonable pathways based on prior knowledge and ecological principles (Fig S5). We applied a stepwise selection procedure to identify the best-fitting model with the lowest Akaike Information Criterion (AIC) using the stepAIC function from the MASS R package. Collinearity between the predictors was avoided, using the variance inflation factor (VIF), by iteratively removing the predictor with the highest VIF from the model and then recalculating the VIF for the remaining predictors until all the VIFs were less than three. The adequacy of the model was assessed through Shipley's test of d-separation: Fisher's C statistic (if $P > 0.05$, then no paths are missing and the model is a good fit) and AIC, implemented in the R package 'piecewiseSEM' [40].

Results

Effects of nitrogen enrichment and warming on bacterial and fungal diversity

To determine whether climate warming and nitrogen enrichment affect soil microbial diversity, we examined the taxonomic diversity (species richness, defined as the number of amplicon sequence variants (ASVs)) of soil bacteria and fungi among treatments in the field experiments via ANOVA analysis. Our results revealed that bacterial diversity differed from fungal diversity in response to N enrichment and warming. Specifically, one-way ANOVA revealed that warming alone did not affect bacterial diversity but significantly decreased fungal diversity by 15.8% ($P < 0.05$, Fig. 1). Low rates of nitrogen enrichment (N1) did not alter the bacterial diversity, whereas high rates of nitrogen enrichment (N2 and N3) significantly decreased bacterial diversity by 8.5% and 10.2%, respectively (all $P < 0.05$, Fig. 1). In contrast, N enrichment increased fungal diversity, with a greater stimulation under higher rates of N enrichment (all $P < 0.05$, Fig. 1). In addition, combined warming and N enrichment decreased bacterial diversity by 1.7%, 11.3%, and 13.5%, respectively, and increased fungal diversity by 27.1%, 37.0%, and 40.7%, respectively, compared with those in the control plot (all $P < 0.05$, except for bacteria in the WN1 treatment; Fig. 1).

Two-way ANOVA revealed that bacterial and fungal diversity were differentially influenced by the interaction between warming and nitrogen addition (all $P < 0.05$, Fig. 1); that is, the effects of warming on microbial

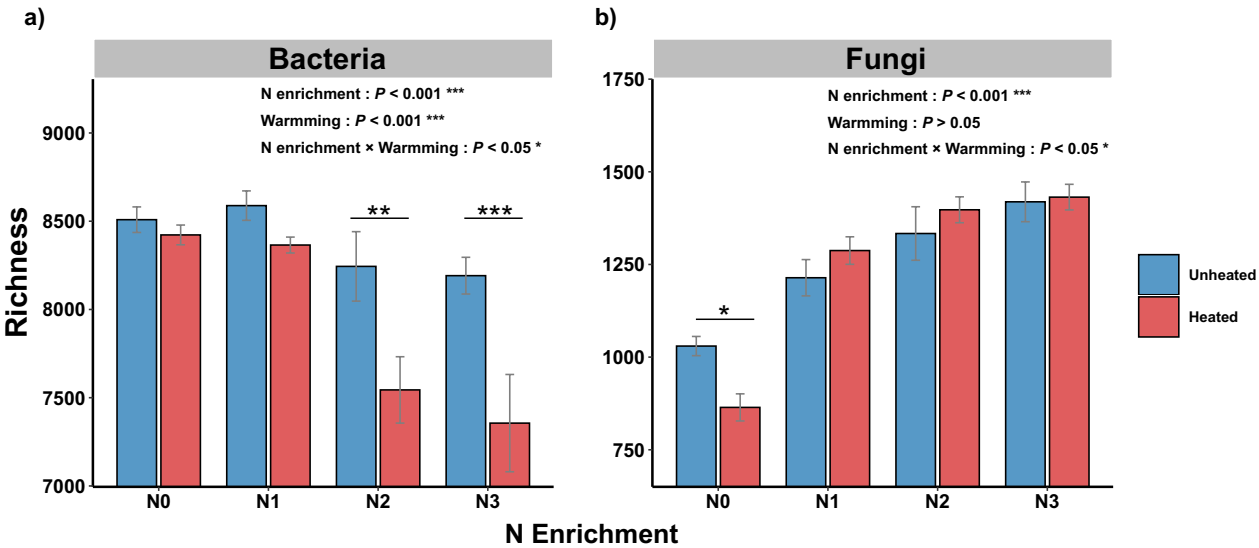

Fig. 1 Effects of experimental warming and N enrichment conditions on soil bacterial and fungal richness. **a**, Effects of warming and N enrichment on bacterial richness. **b**, Effects of warming and N enrichment on fungal richness. N0, N1, N2, and N3 indicate N enrichments of 0, 5, 10, and 15 g m^{-2} yr^{-1}, respectively. Statistical significance was determined by two-way ANOVA followed by Tukey's HSD post hoc test (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). The data are presented as the means ± SEs for six replicate plots for each treatment

diversity varied with the N addition rate. Bacterial diversity was unaffected by warming at relatively low rates of N addition (N0 and N1) but was greatly decreased by warming at high rates of N addition (N2 and N3). However, N addition shifted the effects of warming on fungal diversity from negative to neutral. Therefore, bacterial and fungal diversity responded oppositely to warming, and this response pattern was reversed by N addition.

Effects of N enrichment and warming on bacterial and fungal diversity among different lineages and functional guilds

We further divided the bacterial and fungal taxa into different lineages and functional groups and examined their diversity in response to nitrogen enrichment and warming. The response of the diversity of most microbial phyla to warming and nitrogen enrichment was consistent with that of the kingdom (fungi or bacteria) to which they belong. In contrast, the diversity of Alphaproteobacteria, Actinobacteria, Firmicutes, and Gemmatimonadetes remained unchanged under combined warming and nitrogen enrichment. In addition, warming alone greatly decreased the richness of Ascomycota and Basidiomycota (13.6% – 21.4%, $P<0.01$), but had no effect on the richness of other fungal phyla (Fig. 2).

The richness of the oligotrophic bacteria group remained unchanged under all treatments, whereas the richness of the copiotrophic bacteria significantly increased under nitrogen enrichment (5.3% – 14.7%, $P<0.01$; Fig. 2). The richness of AMF was not affected by warming alone, but significantly increased under nitrogen enrichment and combined with warming (60.7% – 135%, $P<0.01$; Fig. 2). For the richness of the saprotrophic fungi group, warming alone had a significant negative effect (20%, $P<0.01$), while nitrogen enrichment and combined with warming had positive effects (20.5% – 32.1%, $P<0.01$; Fig. 2).

Mechanisms underlying the effects of warming and N addition on bacterial and fungal diversity

We then conducted regression analysis and a piecewise structural equation model (SEM) to identify how changes in biotic interactions and abiotic environmental conditions impact biodiversity under warming and N addition. Regression analysis revealed that the relative abundance of ARGs was negatively correlated with bacterial richness ($P<0.05$, Fig. 3) but positively related to fungal richness ($P<0.05$, Fig. 3).

In addition, SEM revealed that bacterial and fungal diversity are regulated by different environmental drivers. Specifically, soil pH, which was decreased by N enrichment (standardized path coefficient, $b=-0.78$, $P<0.05$; Fig. 4) and warming ($b=-0.25$, $P<0.05$; Fig. 4), played the strongest role in reducing bacterial richness ($b=0.72$, $P<0.05$; Fig. 4). The soil C/N ratio, which was negatively affected by N enrichment ($b=-0.52$, $P<0.05$; Fig. 4), was negatively correlated with fungal diversity ($b=-0.24$, $P<0.05$; Fig. 4). Furthermore, nitrogen application directly affected the fungal richness ($b=0.67$, $P<0.05$; Fig. 4).

Discussion

Assessing the patterns of bacterial and fungal diversity in response to multiple global changes is a significant grand challenge because of the differential sensitivity of these two kingdoms to environmental factors and potential biotic interactions [7, 41]. By examining changes in soil microbial biodiversity in long-term global change experiments, we revealed a decrease in bacterial diversity but an increase in fungal diversity under experimental warming and nitrogen enrichment conditions. Moreover, the negative effects of warming on fungal richness were reversed by N enrichment, and the negative effects of nitrogen enrichment on bacteria were amplified by warming. We also showed that both interkingdom antagonism and shifts in environmental conditions regulated the bacterial and fungal diversity responses. Our findings suggest that fungi might dominate soil carbon and nutrient cycling in a warmer and more nitrogen-enriched world.

Consistent with our hypothesis, we found that combined warming and N enrichment had positive effects on fungal diversity but negative effects on bacterial diversity. In our study, combined long-term nutrient enrichment and warming enhanced the soil organic C quality (reflected by a decreasing soil C/N ratio) (Fig. S1), which is expected to lead to a microbial community with greater bacterial diversity due to the greater capacity of bacteria for resource acquisition [42, 43]. However, our results revealed a reduction in bacterial richness under increased nutrient conditions. This phenomenon may be explained by the fact that soil acidification occurs under nutrient enrichment, which primarily affects bacterial growth and survival and overshadows the benefits of enhanced nutrient availability [44, 45]. Compared with fungi, bacteria must locate more energy sources to alleviate acidity stress, which may subsequently decrease their investment in resource acquisition efforts [46]. Moreover, the optimal pH for the exoenzymes produced by bacteria is neutral or alkaline, whereas fungal exoenzymes perform best at acidic pH [47]. In contrast, owing to greater fungal osmotic stress tolerance capabilities and better performance exoenzymes, fungal growth can be promoted when soil organic C quality is enhanced under acidic conditions. A similar case study in a Qinghai-Tibetan

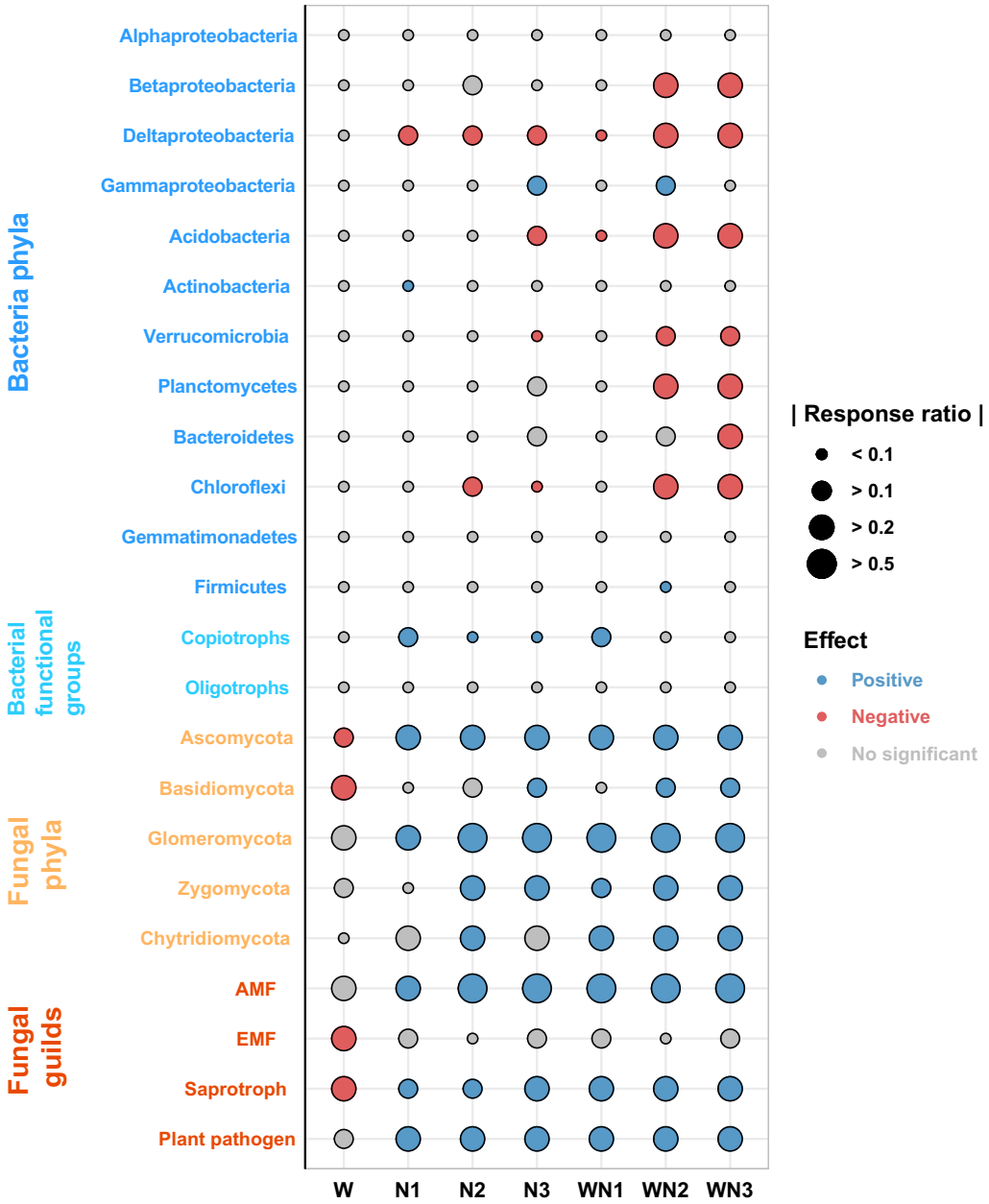

Fig. 2 Effects of warming and N enrichment on the richness of major microbial groups. N1, N2, and N3 indicate N enrichments of 5, 10, and 15 g N m^{-2} yr^{-1}, respectively. W represents the warming treatment. WN1, WN2, and WN3 represent warming combined with N enrichments of 5, 10, and 15 g N m^{-2} yr^{-1}, respectively. Bubble plot illustrating the relative changes in microbial group richness between the treatment and control (N enrichment of 0 g N m^{-2} yr^{-1}). Bubble colour represents the microbial group richness with a significance increase (blue) or decrease (red) under treatment. Bubble size represents the response ratio of microbial group richness under the treatment and control. AMF: arbuscular mycorrhizal fungi; EMF: ectomycorrhizal fungi

plateau alpine steppe shows that three years of nitrogen application and warming had no significant impacts on soil bacterial richness because there were no changes in environmental variables, including soil pH and C/N ratio [48]. Our findings suggest that these soil properties indirectly play a key role in regulating the impacts of nitrogen enrichment and climate warming on soil bacterial and fungal diversity.

The interaction of warming and N enrichment had a synergistic effect on bacterial diversity but an antagonistic effect on fungal diversity. This result may relate to the interactive effects of warming and N addition on

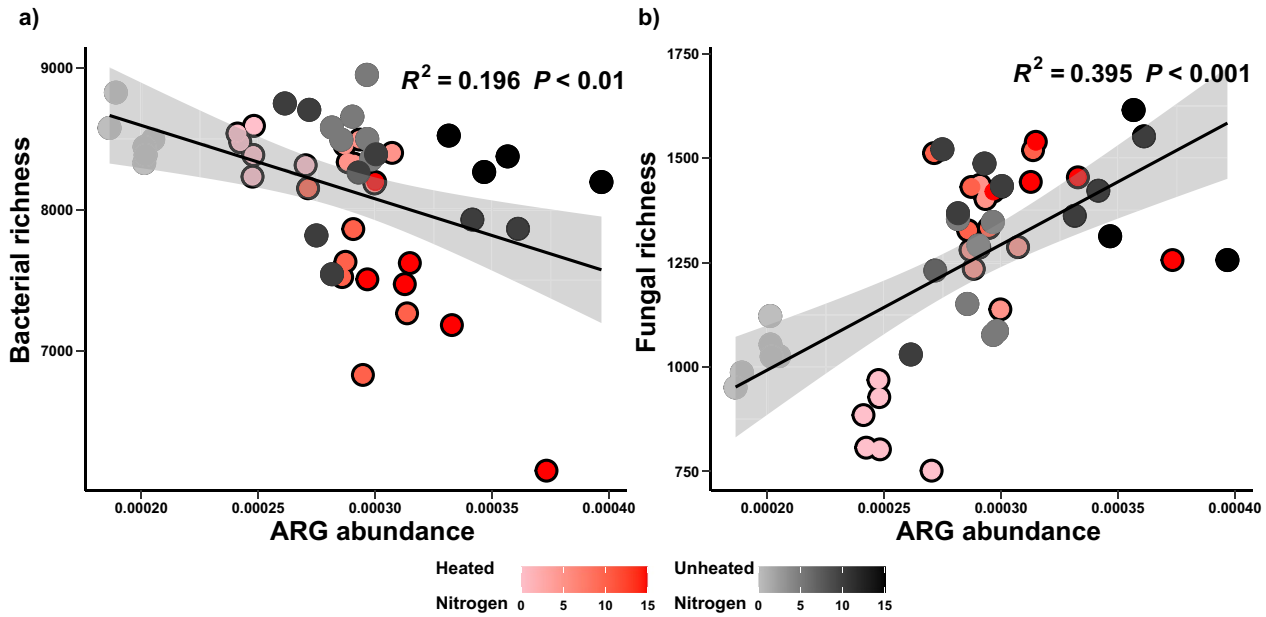

Fig. 3 Effects of the relative abundance of ARGs on soil bacterial and fungal richness. a, Effects of ARG abundance on bacterial richness. b, Effects of ARG abundance on fungal richness. The red colour show heated plots, and the black colour show unheated plots. Saturated colours indicate high rates of N addition, and unsaturated colours indicate low rates of N addition. The solid lines indicate significant relationships ($P < 0.05$). The shaded area is the 95% CI of the estimated relationship

the soil variables. In our study, N enrichment caused soil acidification, and warming further exacerbated soil acidification under high rates of N addition. Moreover, warming increased the soil carbon–nitrogen ratio, but warming did not affect the soil carbon–nitrogen ratio when combined with nitrogen application (Fig. S1). In contrast, a 4-yr field experiment in soda-saline soil in semiarid grasslands revealed that warming and N addition had no independent effects on either bacterial or fungal diversity but interactively reduced soil fungal richness [49], indicating that the interactive effects seem to differ on the base of soil type and N application duration. Our results also revealed that the interactive effects of N enrichment and warming on bacterial and fungal diversity were differentially regulated by the rate of N addition. Under nitrogen enrichment, the negative effect of warming on bacterial diversity intensified with increasing levels of nitrogen addition, whereas the effect of warming on fungal diversity remained unchanged. Elevated temperature may increase nitrifying bacterial activity [22], accelerating NH_4^+ oxidation to NO_3^-, whereas higher nitrogen levels increase NH_4^+ substrates, intensifying nitrification and subsequent H^+ production and exacerbating soil acidification [50]. In contrast, it is possible that nitrogen application may have exceeded nitrogen limitation in ecosystems, and the impact of warming on soil carbon quality under nitrogen sufficiency may be influenced by other factors. Taken together, these results suggest that interactions among multiple global change factors may alter the patterns of bacterial and fungal diversity in response to the individual factors.

In addition to environmental conditions, interkingdom antagonistic interactions also shaped the opposite responses of bacterial and fungal diversity to warming and nitrogen enrichment (Fig. 3). In our study, warming and nitrogen addition significantly increased the relative abundance of ARGs (Fig. S3). The intensified bacterial–fungal antagonism observed may be explained by the significant increase in the relative abundance of Ascomycota under nitrogen enrichment and warming conditions (Fig. S4). Compared with other fungal phyla, filamentous Ascomycota, which are enriched in antibiotic biosynthesis-related gene clusters, have the ability to produce more antibiotics [23]. Our results revealed that the relative abundance of ARGs was significantly negatively correlated with the bacterial richness but positively correlated with fungal diversity (Fig. 3). Similarly, Bahram et al. [23] collected 189 unique topsoil samples covering all terrestrial regions and biomes and reported that bacterial–fungal antagonism determined the distributions of bacterial and fungal diversity. Taken together, these findings indicate that the important role of biotic interactions in shaping the diversity of bacteria and fungi, both in the context of global change and across global geographical scales. Nevertheless, more studies to assess the role of

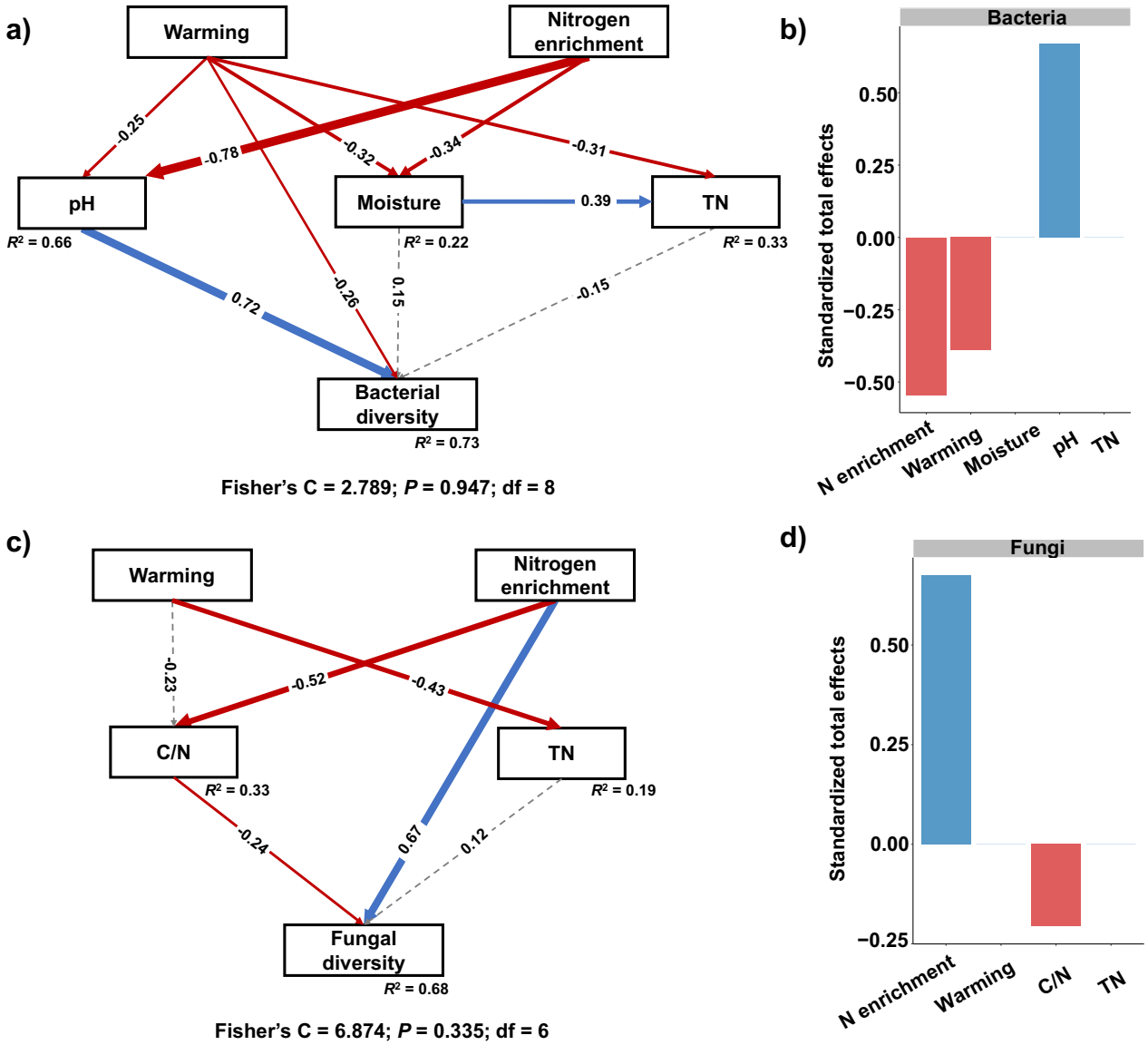

Fig. 4 Structural equation models showing the effects of warming, nitrogen enrichment, and soil variables on microbial diversity. **a**, **c** Path diagrams of factors influencing bacterial and fungal diversity. **b**, **d** Standardized total effects of each driver on bacterial and fungal diversity derived from the model depicted above. The a priori model including all the variables is provided in Fig. S5. The blue and red arrows indicate positive and negative relationships, respectively. The solid or dashed lines indicate significant ($P < 0.05$) or nonsignificant relationships. The numbers near the pathway arrows indicate the standard path coefficients. R^2 represents the proportion of the variance explained for each variable in the model. The goodness-of-fit statistics for the models ($P > 0.05$) indicate a close fit of the models to the data. C/N, soil carbon: nitrogen. TN, soil total nitrogen

interkingdom antagonism in microbial diversity under diverse global change drivers are needed in the future.

Another major finding of our study is that the effects of N enrichment and warming on bacterial and fungal diversity varied among different lineages and functional guilds (Fig. 2). Specifically, the richness of most bacterial phyla significantly decreased, such as those of Deltaproteobacteria and Acidobacteria, whereas the richness of all fungal phyla increased under nitrogen addition and warming. Deltaproteobacteria and Acidobacteria are very sensitive to elevated nutrients, which was revealed in a previous nutrient input study conducted at 25 globally distributed grassland sites [28]. In contrast, the diversity of Firmicutes and Actinobacteria was only slightly affected by N addition and warming, which may be attributed to their spore-forming ability, which renders them

resistant to abiotic stress. Our data indicate that nitrogen enrichment and warming have no discernible effect on the richness of copiotrophic bacterial groups but significantly increase their relative abundance, which is consistent with previous studies suggesting that increased nutrient availability benefits the growth of copiotrophs [28] (Fig. 2; Fig. S4). Additionally, the observed increase in the richness of arbuscular mycorrhizal fungi (AMF) under nitrogen enrichment and warming in our study may be explained by the stimulation of hyphal growth or the promotion of plant growth and subsequent carbon allocation to AMF [51, 52].

On the basis of the large increase in fungal diversity and decrease in bacterial diversity under nitrogen enrichment and experimental warming conditions in our study, we speculate that soil nutrient cycling ecosystem functions are driven primarily by fungi in a warmer and nitrogen-enriched world. This speculation is supported by the results of previous studies investigating the relationships between microbial diversity and ecosystem functions. For example, a 2-year field experiment in a temperate forest revealed that canopy N addition enhanced soil ecosystem multifunctionality, which was regulated mainly by the increased fungal diversity [53]. Similarly, Anthony et al. [54] investigated the relationship between soil microbial diversity and reported that increased fungal but not bacterial richness was tightly coupled with increased tree growth rates and biomass carbon stocks. Moreover, the diversity of AMF under warming and nitrogen enrichment increased, indicating a potential increase in aboveground plant productivity and overall plant diversity [55, 56]. Additionally, under nitrogen enrichment and warming, a large increase in the relative abundance of copiotrophic bacteria occurs, which may slow soil carbon loss due to the lower resource use efficiency and greater thermal adaptation capacity of copiotrophs than oligotrophs [57]. Given the beneficial effects of increased fungal and arbuscular mycorrhizal fungal (AMF) diversity on ecosystem functions, the perceived severity of the impacts of nitrogen enrichment and climate warming on microbial diversity may have been overstated [9, 58, 59]. Therefore, by simultaneously assessing bacterial, fungal, and functional group diversity under multiple climate change scenarios, we can more accurately predict microbial diversity and associated ecosystem functions in the future.

Conclusion

Our findings revealed opposite response patterns of bacterial and fungal diversity to combined warming and N enrichment, providing important implications for predicting the relative contributions of these two kingdoms to ecosystem functions in a warmer and nitrogen-enriched world. Given the increased diversity of fungi and AMF groups and the reduced bacterial diversity under warming and nitrogen enrichment, it is expected that ecosystem functions driven by fungi, such as soil carbon sequestration, plant diversity, and productivity [56, 60], are likely to be significantly enhanced in the future. As nitrogen enrichment changed the response pattern of bacterial and fungal diversity to warming in our experiments, our findings emphasize the need to consider the interactive effects of multiple global change factors on soil microbial diversity. However, caution should be exercised when generalizing our findings to other ecosystems, as our research was specifically conducted on the Tibetan Plateau, a region highly vulnerable to climate warming and typically nitrogen-constrained [61, 62]. Further study is necessary to determine whether the contrasting response patterns of bacterial and fungal diversity and their associated mechanisms, are consistent across other ecosystems under multiple global change factors.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-025-00693-7.

Additional file 1.

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Author contributions
M.N. conceptualized the study; X.L. and S.Z. helped to provide the samples. H.L., X.X. and J.X. performed the analyses; J.X. wrote the manuscript with the help from all co-authors.

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Availability of data and materials
The Raw sequences of amplicons and metagenomes are deposited in the National Center for Biotechnology Information (NCBI) under project accession number PRJNA1122351 and PRJNA1122921, respectively.

Declarations

Ethics approval and consent to participate
This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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