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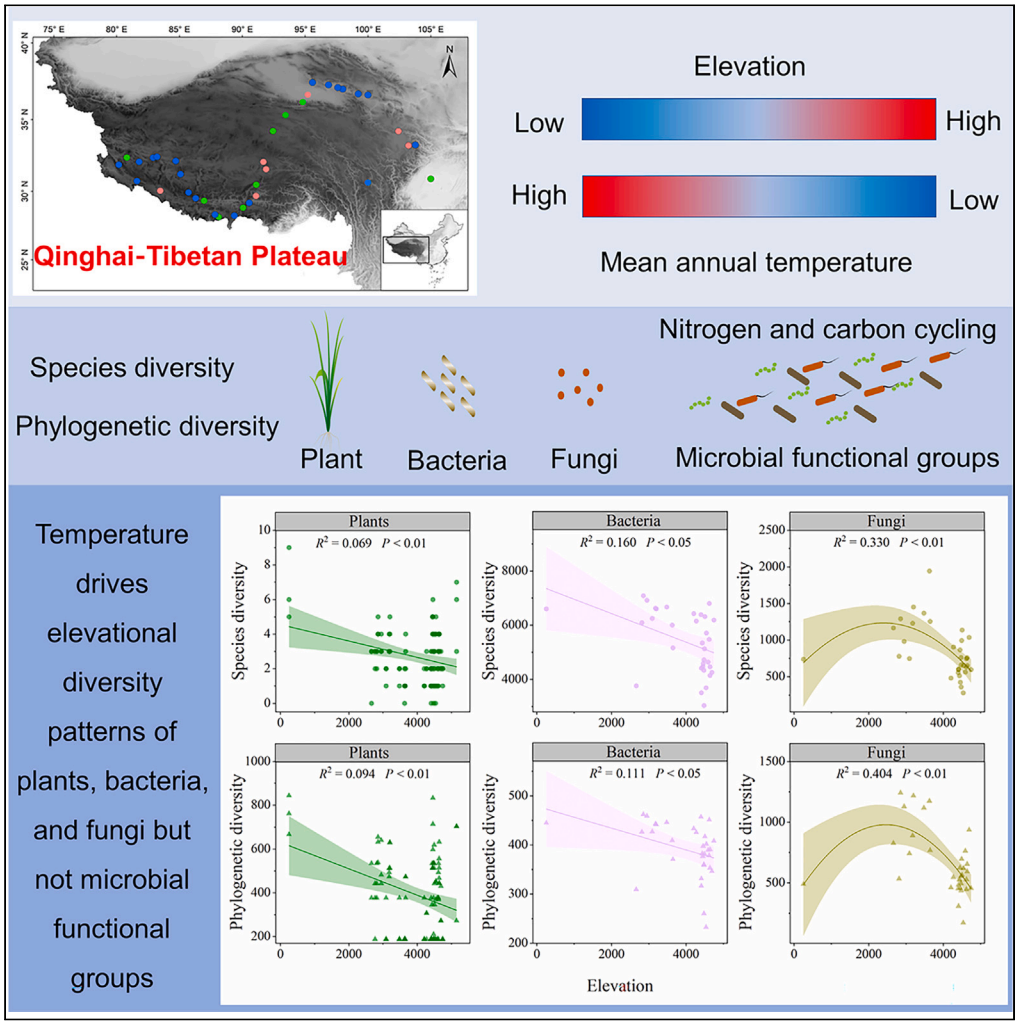

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Highlights

Diversity of plants, bacteria, and fungi exhibited a significant elevational gradient

No significant diversity changes observed for microbial functional groups

Temperature drives elevational diversity patterns of plants, bacteria, and fungi

Species and phylogenetic diversity of plants and fungi varied with wetland type

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Temperature drives elevational diversity patterns of different types of organisms in Qinghai-Tibetan Plateau wetlands

Bangjing Ding,[1,5] Lian Feng,[2,3,5] Sang Ba,[2,3] Xiaoliang Jiang,[4] Guihua Liu,[1] and Wenzhi Liu[1,6,*]

SUMMARY

The spatial pattern and driving mechanism of biodiversity along elevational gradients are key topics in ecology. However, it is still unclear whether the multidimensional diversity of different types of organisms shows a similar response to elevation changes. Here, we measured the species and phylogenetic diversity of plants, bacteria, fungi, and microbial functional groups (nitrifiers, denitrifiers, methanogens, and methanotrophs) in 36 wetland sites on the Qinghai-Tibetan Plateau. The results showed that both species and phylogenetic diversity of plants, bacteria, and fungi exhibited a significant elevational gradient, in direct contrast to no significant diversity changes observed for denitrifiers, methanogens, and methanotrophs along the same altitude gradient. Our findings suggest that elevation and temperature were more likely to associate with the diversity of plants, bacteria, and fungi than the diversity of microbial functional groups, with important implications for assessing the effect of ongoing climate warming on biodiversity in Qinghai-Tibetan alpine wetlands.

INTRODUCTION

One of the fundamental topics in ecology is to explain why there are many different types of organisms on the living Earth.[1] The spatial pattern of species diversity has long been a main focus of ecologists, biogeographers, and conservation biologists in the past century.[2–4] Species diversity is a basic ecological feature of biological communities and is commonly associated with the productivity, stability, complexity, invasiveness, and functioning of both terrestrial and aquatic ecosystems.[5,6] Over the past few decades, scientists have recognized that multiple aspects of biodiversity, such as phylogenetic diversity, should be considered because they can provide complementary information to enhance our understanding of the mechanisms shaping biodiversity patterns.[7] Phylogenetic diversity describes the phylogenetic distance and evolutionary relationship between co-occurring species in a community and has received rapidly increasing attention from biodiversity researchers and managers in recent years.[8–10]

Both species diversity and phylogenetic diversity are influenced by a wide variety of biotic and abiotic factors.[11,12] At large spatial scales, elevation has been proven to be a critical abiotic factor in determining the spatial pattern of diversity in plant, animal, and microbial communities.[3,13] In general, macroorganisms such as herbs, reptiles, and amphibians exhibit either monotonically decreasing or hump-shaped diversity patterns with increased elevation.[14,15] However, some recent studies have reported that microorganisms, including bacteria, fungi, and protists, do not follow the typical diversity pattern of macroorganisms across the elevation.[3,16] The increasing elevation is commonly linked with a decline in temperature and changes in other environmental characteristics, such as precipitation, air humidity, and soil formation processes.[15]

The elevational pattern of species and phylogenetic diversity for macroorganisms has been well documented in the literature.[17–19] Some studies have also examined the relationships between the species diversity of soil microorganisms and elevation.[14,18,20] However, there are still limited studies investigating how the phylogenetic diversity of soil microbes varies across elevational gradients, especially in high-altitude and cold areas where surface soils may be frozen for several months each year.[21,22] This represents a significant gap in our understanding of biodiversity patterns because microorganisms in soils are extremely diverse and abundant globally and play a crucial role in regulating numerous ecological processes and

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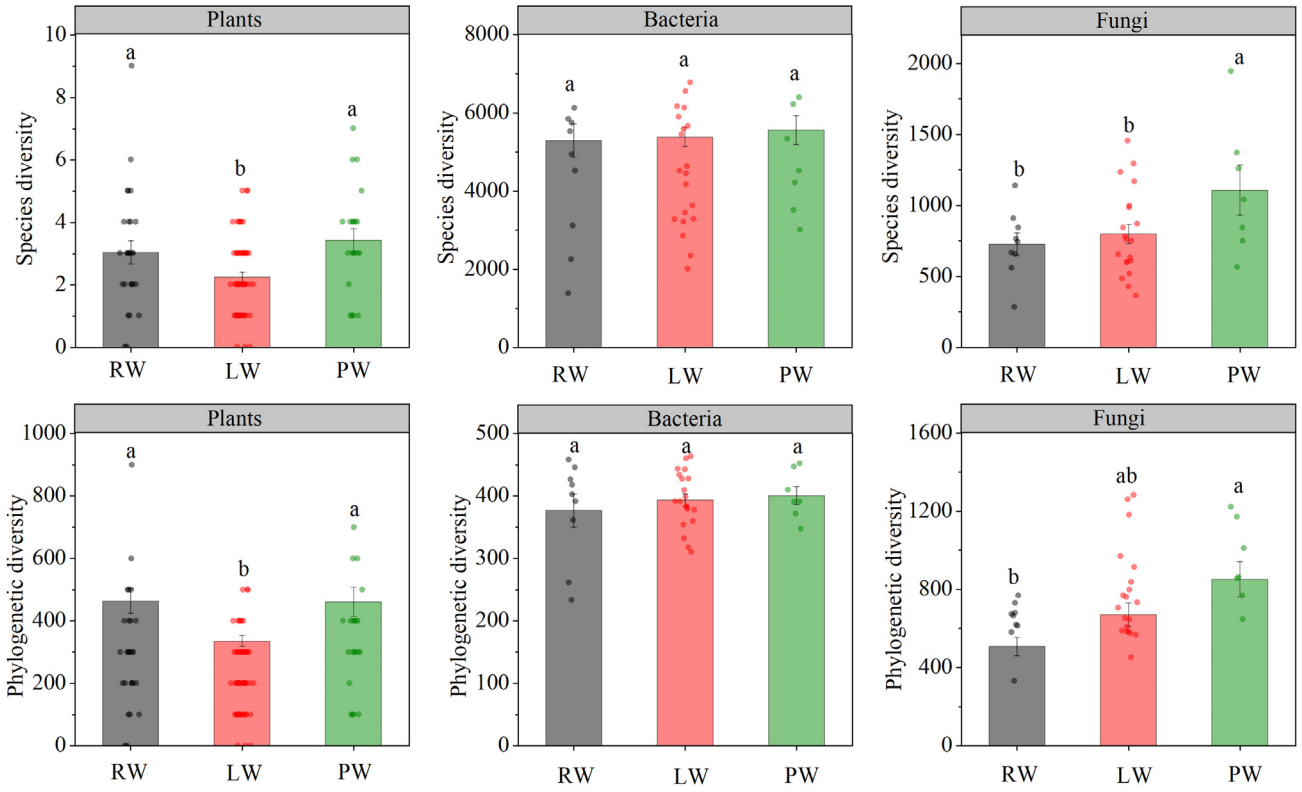

Figure 1. Species and phylogenetic diversity of plants, bacteria, and fungi in different wetland types

The different small letters above the different columns denote significant differences between the three wetlands ($p < 0.05$). RW, LW, and PW represent riverine, lacustrine, and palustrine wetlands, respectively.

functions.[23] Therefore, more studies are needed to better understand the elevational pattern of phylogenetic diversity for soil microbes and their underlying mechanisms.

The Qinghai-Tibetan Plateau is known as the "Third Pole", with an average altitude of more than 4000 m. This plateau contains a large area (approximately 13.2×10^4 km^2) and diverse wetlands which support a rich and unique biota.[24] In this study, we investigated the species and phylogenetic diversity of plants and soil microbes (bacteria, fungi, nitrifiers, denitrifiers, methanogens, and methanotrophs) in 36 riverine, lacustrine, and palustrine wetland sites on the Qinghai-Tibetan Plateau. The objectives of our study were to test three hypotheses: (1) species and phylogenetic diversity of plants and soil microbe vary with wetland type (i.e., riverine, lacustrine, and palustrine); (2) species and phylogenetic diversity of both plants and soil microbes decrease with increasing elevation; (3) the effects of elevation on species and phylogenetic diversity of plants and soil microbes are mainly mediated through changes in temperature and precipitation.

RESULTS

Species and phylogenetic diversity of plants and soil microbes

Plant species diversity in plots ranged from 0 to 9 species (Table S3), which varied significantly with wetland type (Figure 1). Plant phylogenetic diversity varied between 188.3 and 844.4 (Table S4), and a significant difference in plant phylogenetic diversity was also observed with respect to wetland type (Figure 1). Bacterial species and phylogenetic diversity varied from 3039 to 7085 and from 232.6 to 462.6, respectively (Tables S3 and S4), whereas both species diversity and phylogenetic diversity of bacteria corresponding to the three wetland types were not significantly different (Figure 1). By contrast, both species diversity and phylogenetic diversity of fungi showed significant variations with wetland type (Figure 1). Additionally, both species diversity and phylogenetic diversity of microbial functional groups showed nonsignificant differences with wetland type, except for AOB (Figure S4). Interestingly, we found that species diversity and

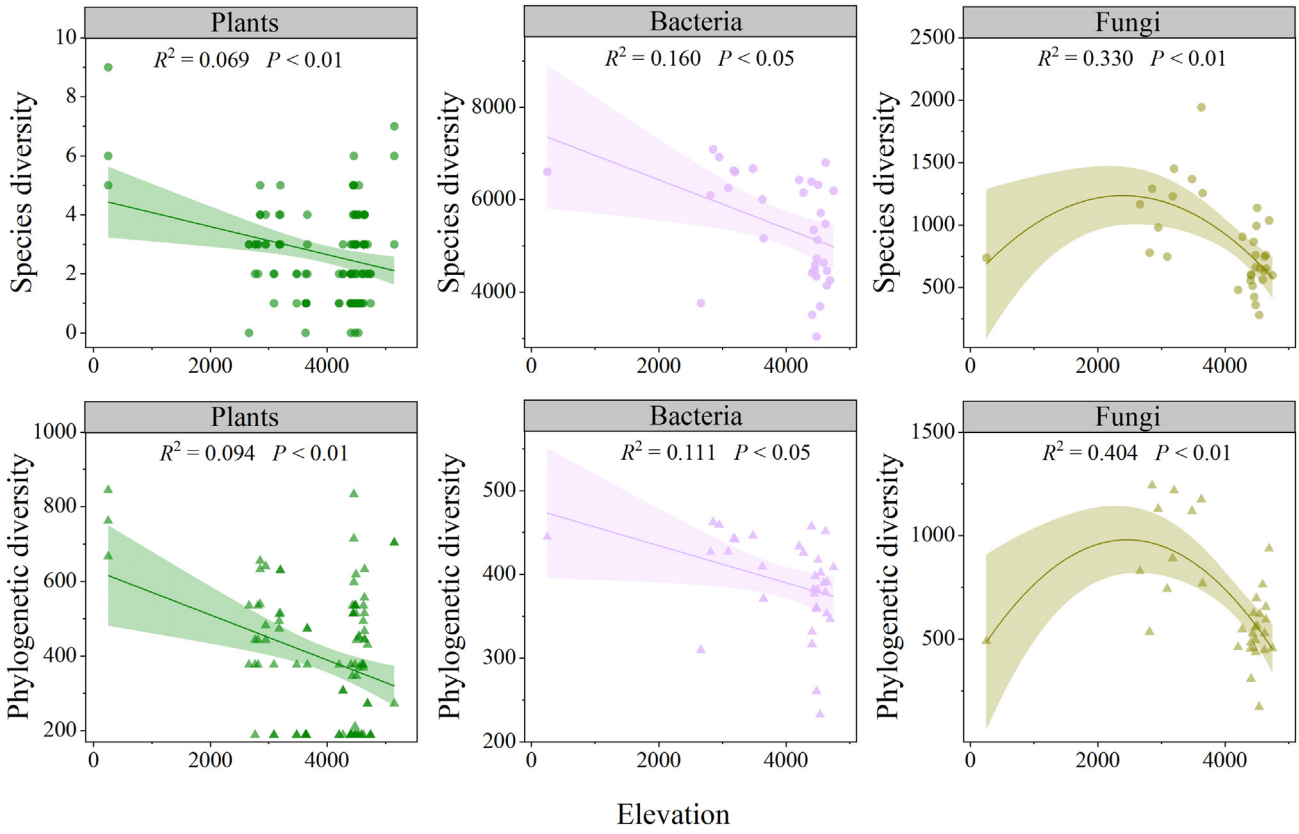

Figure 2. Changes in plant, bacterial, and fungal diversity across the elevational gradient

The solid lines and confidence intervals show predicted relationships and 95% confidence intervals from polynomial models, respectively.

phylogenetic diversity of plants and/or soil microbes exhibited parallel patterns, except for *nirS*-type denitrifiers, methanogens, and methanotrophs (Table S5).

Patterns of biodiversity along an elevational gradient

As expected, there were clear changes in the diversity of plants, bacteria, and fungi along an elevational gradient (Figure 2; $p < 0.05$ in all cases). For plants, both species diversity and phylogenetic diversity showed the steepest decline with elevation, and the decline was described with a linear model (Figure 2). For bacteria, the change was also linear, whereas for fungi, both species diversity and phylogenetic diversity were the highest at mid-elevation with a unimodal pattern (Figure 2). For microbial functional groups, the diversity had a significant difference in the exact pattern of change with elevation (Figure 3). The diversity of nitrifiers along an elevational gradient was described with non-linear model and was lowest at mid-elevation, except for species diversity of AOB (Figure 3). Furthermore, we found no evidence for an elevational gradient in the diversity of denitrifiers, methanogens, and methanotrophs as there was no significant relationship between elevation and species diversity or phylogenetic diversity within each microbial functional group (including denitrifiers, methanogens, and methanotrophs), regardless of the model used (first-order or second-order polynomial; $p > 0.05$ in all cases). Being different from biodiversity, the abundance of plants and soil microbes showed no significant correlation with elevation (Table S6).

Relationships between biodiversity and environmental factors

Species diversity and phylogenetic diversity of plants increased significantly with mean annual temperature (MAT) and mean annual precipitation (MAP) (Figure 4). Bacterial and fungal diversity were also positively affected by MAT, but they did not demonstrate a significant relationship with MAP (Figure 4). The biodiversity of plants, bacteria, and fungi tended to decrease with soil pH in plateau wetlands (Figure 4). Furthermore, bacterial and fungal species diversity significantly increased with soil NH_4^+ content, and bacterial phylogenetic

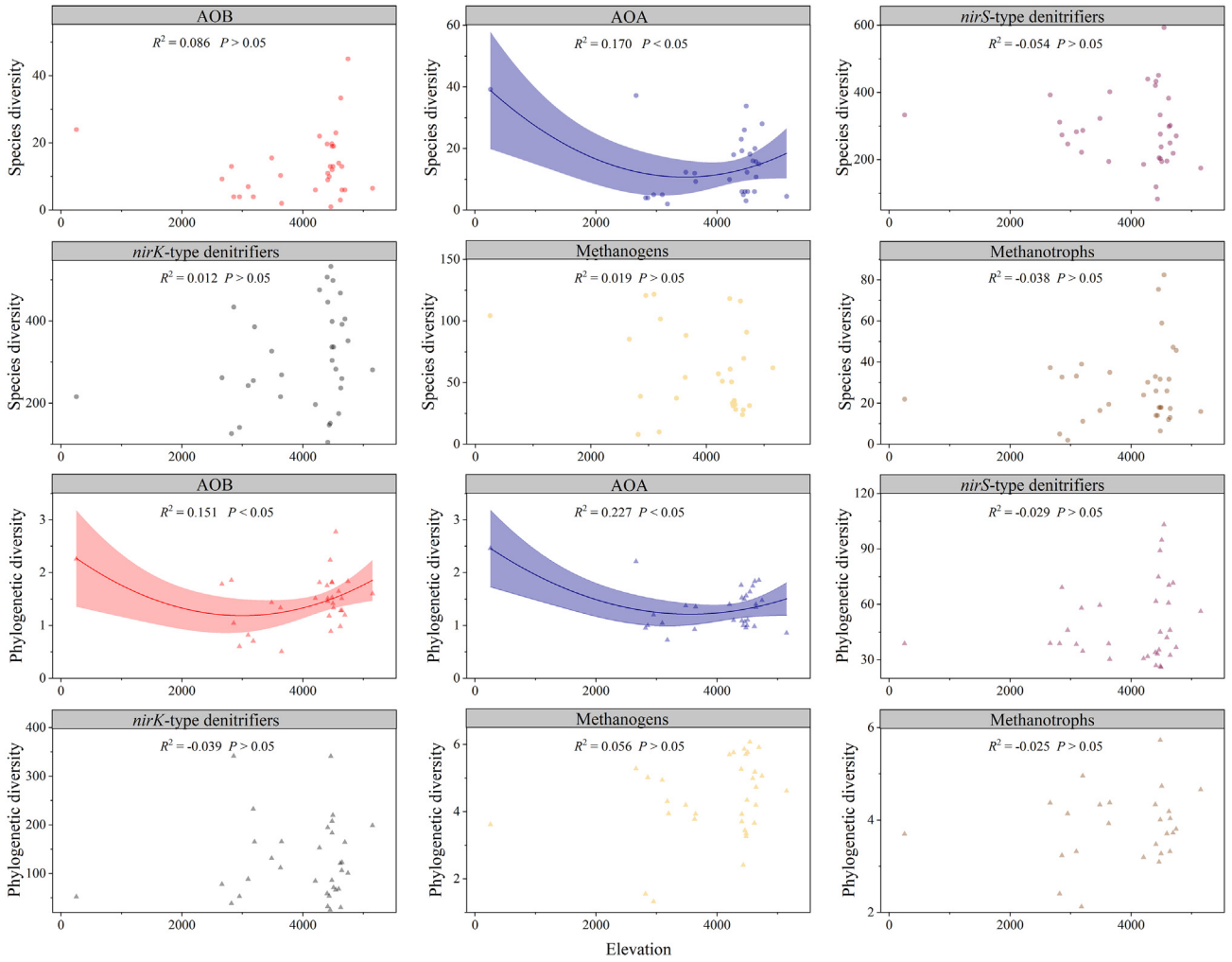

Figure 3. Changes in diversity of microbial functional groups across the elevational gradient

The solid lines and confidence intervals show predicted relationships and 95% confidence intervals from polynomial models, respectively.

diversity also increased with soil moisture and NH_4^+ content (Figure 4). However, there was no significant relationship among the biodiversity of plants, bacteria, and fungi and soil temperature, conductivity, total carbon (TC) content, NO_3^- content, and TP content. With regard to microbial functional groups, their biodiversity could also be regulated by relevant environmental factors (Figure 4). Soil conductivity was significantly related to species diversity of nitrifiers (AOB and AOA) and phylogenetic diversity of AOA. Moreover, both soil temperature and NO_3^- content were associated with the species diversity of *nirK*-type denitrifiers, while both soil moisture and TC were associated with the phylogenetic diversity of *nirS*-type denitrifiers. Additionally, MAP was related to the species diversity of methanogens, and soil temperature was related to the species diversity of methanotrophs, whereas no significant relationship was observed between environmental factors and phylogenetic diversity of methanogens or methanotrophs.

Path analysis of elevation effects on biodiversity

For the model of plant species diversity, elevation was identified as the strongest predictor explaining plant species diversity (Figures 5 and S5). In the model, elevation, MAT, MAP, pH, and wetland type together explained 37.4% of the variance in plant species diversity (Figure 5). For the model of bacterial species diversity, elevation and soil NH_4^+ were the dominant factor explaining bacterial species diversity, whereas they had the opposing effects on bacterial species diversity (Figure S5). 23.0% variations in the bacterial species diversity could be explained by elevation, soil NH_4^+, MAT, MAP, and wetland type (Figure 5). For the model of fungal species diversity, elevation, soil pH, and wetland type had relatively large effects

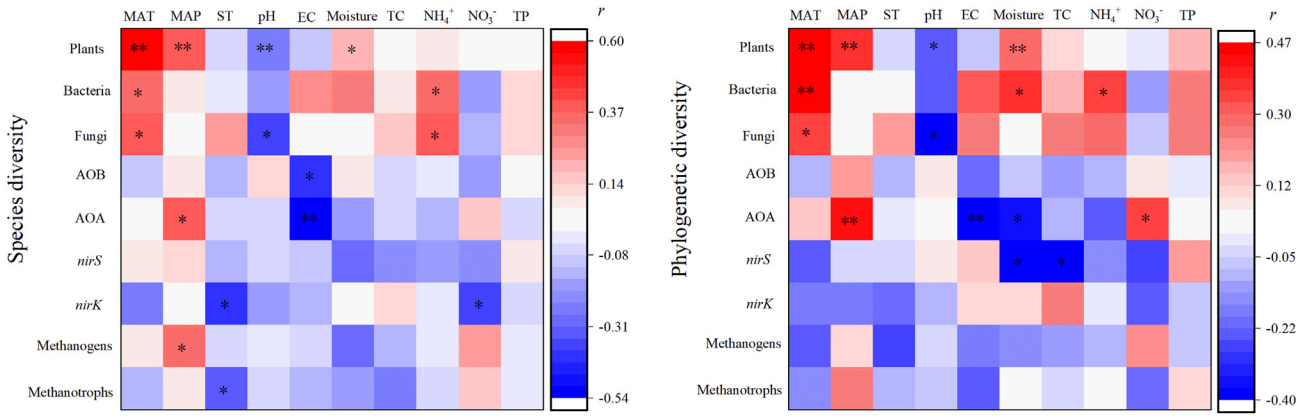

Figure 4. Pearson correlation coefficients between species diversity and phylogenetic diversity and environmental parameters

Asterisks indicate significant influence (** indicates $p < 0.01$; * indicates $p < 0.05$). ST: soil temperature; EC: conductivity; TC: total carbon; *nirS*: *nirS*-type denitrifiers; *nirK*: *nirK*-type denitrifiers.

on fungal species diversity (Figure S5). This model explained 39.3% of the variation in the fungal species diversity by elevation, wetland type, and environmental factors (Figure 5).

In the model of plant phylogenetic diversity, elevation and MAT could play a primary role in regulating plant phylogenetic diversity (Figure 5). MAT had the strongest direct effect on plant phylogenetic diversity, while elevation had the strongest indirect effect (Figure S5). The final models accounted for 26.4% of the variation in plant phylogenetic diversity (Figure 5). For the model of bacterial phylogenetic diversity, MAT was the dominant factor explaining bacterial phylogenetic diversity, but we did not find significant direct effects of elevation on bacterial phylogenetic diversity (Figures 5 and S5). SEM showed that elevation, climatic factors, wetland type, and soil physicochemical properties could explain 34.3% variations in bacterial phylogenetic diversity (Figure 5). For the model of fungal phylogenetic diversity, wetland type exhibited primary and positive effects on fungal phylogenetic diversity, whereas elevation and pH showed a relatively large negative effect on fungal phylogenetic diversity (Figures 5 and S5). 39.9% of the variation in fungal phylogenetic diversity could be explained by wetland type, elevation, pH, MAT, and MAP (Figure 5).

With regard to microbial functional groups, the models showed that elevation and MAT had a weak influence on species diversity and phylogenetic diversity (Figure S6 and Table S7). These models explained 26.2%, 36.6%, 19.1%, 28.8%, 22.0%, and 12.5% of the variation of species diversity and 25.0%, 28.8%, 20.6%, 11.8%, 14.3%, and 15.5% of the variation of phylogenetic diversity in AOB, AOA, *nirS*-type denitrifiers, *nirK*-type denitrifiers, methanogens, and methanotrophs, respectively (Figure S6). The model of AOB provided evidence that wetland type exhibited a primary role in the diversity of AOB (Figure S6 and Table S7). Results also showed that MAP had a relatively strong effect on species diversity and phylogenetic diversity of AOA (Table S7). Additionally, TC was the dominant factor explaining variations in the phylogenetic diversity of denitrifiers, while MAP was the dominant factor explaining variations in the species diversity of methanogens and phylogenetic diversity of methanotrophs (Table S7).

DISCUSSION

Contrasting elevational patterns of plant and microbial diversity

As we hypothesized, this study found that both species and phylogenetic diversity of vascular plants in Qinghai-Tibetan wetlands declined with increased elevation (Figure 2). The relationship between plant diversity and elevation has long been a fundamental topic of ecologists and biogeographers for two centuries.[25–27] Previous studies found that plants exhibit either monotonically decreasing or hump-shaped richness patterns with elevation.[3,14,16,20] For example, Vázquez and Givnish reported that understorey herbs, shrubs, and vines in tropical forests of Mexico showed the greatest decline in species richness with increasing altitude.[28] Wang et al.[29] indicated that the richness of seed plants at species, genus, and family levels in the Gaoligong Mountains of China all showed hump-shaped patterns along the altitudinal gradient. In total, elevational patterns of diversity for plants and other macroorganisms (e.g., mammals, birds, and amphibians) have been well established.

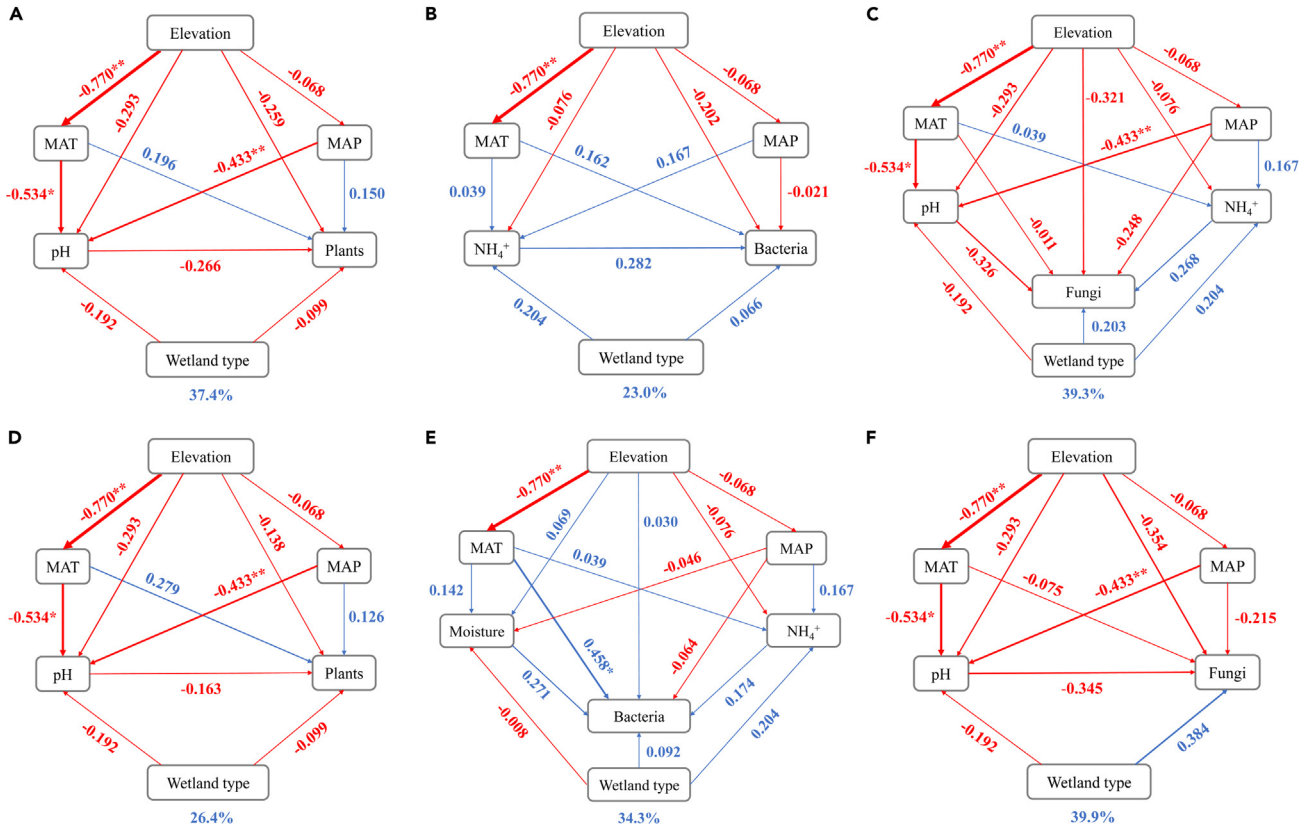

Figure 5. Structural equation models of species diversity and phylogenetic diversity in plants, bacteria, and fungi

(A–F) The species diversity of plants (A), bacteria (B), and fungi (C), respectively, and the phylogenetic diversity of plants (D), bacteria (E), and fungi (F), respectively. The red and blue arrows represent negative and positive pathways, respectively, while the numbers adjacent to the arrows represent standardized path coefficients, analogous to relative regression weights and indicative of the size effect of the relationship. The arrow width is proportional to the strength of the relationship. ** and * denote statistical significance at $p < 0.01$ and $p < 0.05$, respectively.

By contrast, we know relatively little about how microbial diversity varies across elevational gradients because of limited technology in measuring diversity accurately in the past.[14] As expected, we found that bacteria in Qinghai-Tibetan wetlands exhibited an elevational gradient in diversity similar to that observed for plants (Figure 2). However, the elevational pattern of fungal diversity in Tibetan wetlands showed a unimodal pattern, peaking at mid-elevation (Figure 2). Fierer et al.[3] found that there was no significant relationship between elevation and species richness or phylogenetic diversity of soil bacteria in the eastern Andes of Peru, regardless of the model used. Shen et al.[16] also indicated that the diversity of bacteria and fungi along Changbai Mountain in China did not correlate with elevation. However, some studies reported inconsistent results. For instance, Bryant et al.[14] first reported that the species richness and phylogenetic diversity of bacteria within the phylum *Acidobacteria* decreased monotonically from the subalpine to the alpine in the Colorado Rocky Mountains. Nottingham et al.[20] further found that the α-diversity of both bacteria and fungi was inversely correlated with the elevation in the Peruvian Andes. Diversity gradients were gentler for bacteria and fungi compared to plants, which was in accordance with a viewpoint that microorganisms were more diverse and widespread than plants.[20] The microbial results presented here, together with previous studies, suggest that for both soil bacteria and fungi, responses of diversity to altitude are not regulated by the same mechanisms in different environments.

Until now, there has been a paucity of studies examining the response of microbial functional groups involved in N and C cycles to elevation. To our knowledge, this study is the first attempt to compare elevational biodiversity patterns among plants, bacteria, fungi, and microbial functional groups. Here, we found that soil denitrifiers, methanogens, and methanotrophs in Qinghai-Tibetan wetlands exhibited nonsignificant elevational gradient in diversity (Figure 3). The lack of apparent elevational gradient in the diversity of microbial functional groups contrasts with the results reported by Zhang et al.,[30] who reported that AOA

but not AOB in river sediments of the Qinghai-Tibet Plateau significantly decreased with increasing elevation. *In an earlier study*, Singh et al.[31] observed that different subgroups (or phyla) of soil bacteria on Mountain Fuji showed distinct elevational patterns in species and phylogenetic diversity. It is not surprising that different subgroups of bacteria show quite distinct trends because they are often genetically *distinct from one another*. Moreover, these differences may be caused by different pH sensitivities, nutrient affinities, carbon substrate preferences, and oxygen tolerances of microbial functional groups. Our results clearly showed that the responses of functional microbes in soils to elevational gradients are not always consistent, and the mechanism that shapes these patterns is still the subject of active debate.

Mechanisms underlying elevational pattern of plant and microbial diversity

Elevation is a complex variable that represents the combined effects of numerous environmental variables important for plant growth.[3] The increasing elevation is generally associated with not only a decline in air temperature but also a decline in land area and changes in other environmental factors.[15,32] In this study, we found that elevation was inversely correlated with air temperature but not with precipitation and soil physicochemical properties on the Tibet Plateau (Figure S7). This result may imply that the indirect effect of elevation on the diversity of plants and soil microbes was primarily mediated through air temperature.

MAT exhibited positive relationships with the diversity of plants, bacteria, and fungi (Figure 4). The relationship between air temperature and plant diversity has been well established. Plant community shifts along elevational gradients have long been thought to correspond to temperature changes and comprise a classic biogeographic pattern in both tropical and temperate zones.[20] However, the strong positive associations between temperature and the diversity of plants, bacteria, and fungi were not detected for microbial functional groups (Figure 4). These results may imply that the hypothesis relating diversity pattern directly or indirectly to the temperature dependence of organism metabolism cannot apply to all microbial groups.[3] On the one hand, it is well known that different organisms have different temperature ranges for growth, resulting in different responses to temperature fluctuations.[33] On the other hand, the effects of temperature on soil microbial diversity could be indirectly influenced by plant diversity, with which temperature is strongly correlated. Zhou et al.[34] reported that higher plant richness caused by higher temperatures could provide more nutrients and substrates to soil microorganisms, leading to higher microbial diversity. Here, we found that plant diversity was strongly related to bacterial and fungal diversity but not to the diversity of microbial functional groups (Table S5). We also found that MAT was positively correlated with the abundance of plants but not soil microbes (Table S6). However, Chen et al.[35] reported that MAT was positively related to the abundance of AOA nitrifiers and *nirS*-type denitrifiers in soils of Tibetan grasslands. In addition, through an incubation experiment, Waghmode et al.[36] revealed that warming increased the abundance of AOB and decreased the abundance of AOA and denitrifier (*nirK*, *nirS*, and *nosZ*).

The water-energy hypothesis predicts that sites with higher precipitation and evapotranspiration support more species.[37] It has been recognized that the paradigm that applies to macroorganisms does not necessarily hold for microorganisms and that water availability is not directly related to the diversity of all organisms.[38] Little is known about the variation in microbial diversity along precipitation gradients at a large scale. In this study, we found that MAP was uncorrelated with the diversity of soil microbes except for the diversity of AOA and species diversity of methanotrophs (Figure 4). Bachar et al.[38] reported that soil bacterial abundance decreases with precipitation, and bacterial diversity was independent of precipitation gradient. However, our results indicated that MAP did not correlate with the abundance of plants and soil microbes (Table S6), in contrast to previous findings highlighted that increased MAP significantly increased the gene abundance of nitrification and denitrification in a desert steppe.[39] This result implies that the local soil environmental conditions are much more important than MAP in determining the abundance of plants and soil microbes in the plateau wetlands.

SEM revealed that elevation directly and indirectly drove variation in plant, bacterial, and fungal diversity, highlighting the important effects of elevation. Given that temperature and rainfall usually change gradually with increasing elevation,[15,40] it is not surprising to find direct effects of elevation on plant, bacterial, and fungal diversity in Tibetan wetlands. Elevation had a significant and direct effect on MAT, which could regulate biodiversity both directly and indirectly (Figure 5). MAT had a relatively large effect on the diversity of plants, bacteria, and fungi, whereas a relatively small effect on microbial functional groups. In contrast to MAT, elevation had a very weak direct effect on MAP (Figure 5), indicating that the indirect effect of

elevation on biodiversity was mainly mediated by MAT rather than MAP. Additionally, our models showed that the wetland type exhibited a relatively strong effect on regulating changes in the diversity of fungi and AOB, mainly through direct effects (Figure S6 and Table S7).

Effects of soil physicochemical properties on plant and microbial diversity

In addition to the main effect of MAT, there was a secondary role for other environmental and edaphic properties in shaping these diversity patterns.[20] The importance of pH influencing biodiversity patterns has been well demonstrated. For example, a previous study showed a significant and negative relationship between fungal diversity and soil pH in highly saline grassland soils.[41] Partel et al.[42] reported that pH could regulate changes in plant diversity in Northern Europe. Our results demonstrated that soil pH played a relatively large role in predicting the variation of plant and fungal diversity (Figures 5 and S5). However, soil pH was not a strong predictor of the diversity of bacteria and microbial functional groups in the plateau wetlands, in agreement with the study by Fierer et al.,[3] who indicated that soil pH was not a strong predictor of bacterial diversity along an elevational gradient in the eastern Andes of Peru.

Besides pH, soil physiochemical properties such as conductivity were correlated with the diversity of nitrifiers (Figure 4). Soil conductivity can reflect the salinity, and many palustrine and lacustrine wetlands on the Qinghai-Tibetan Plateau are saline.[43] In this study, soil conductivity was considered an important factor driving the variation of the ammonia-oxidizing microbial community. Available studies demonstrated that soil conductivity was proposed to be the main environmental factor affecting the diversity of nitrifiers in the Ebinur Lake wetland.[44] Zhou et al.[45] pointed out that soil conductivity influenced AOB community composition in wetlands on the Qinghai-Tibetan plateau. Our models demonstrated that soil conductivity was the primary factor driving the variation of the species diversity but not the phylogenetic diversity of AOA and AOB (Table S7). Thus, soil conductivity could play an important role in the species diversity of nitrifiers.

In conclusion, this study revealed the overall pattern of the diversity of different microbial groups along an elevation gradient. Our findings demonstrate that microbial functional groups (denitrifiers, methanogens, and methanotrophs) do not follow the elevational diversity pattern of plants, bacteria, and fungi. Furthermore, our findings illustrate that the effects of elevation on species and phylogenetic diversity of plants and soil microbes are mainly mediated through changes in temperature rather than precipitation. These findings highlight the importance of elevation and temperature in driving the diversity of plants, bacteria, and fungi but not soil microbial functional groups in Qinghai-Tibetan alpine wetlands. Overall, our results fill a critical gap in our understanding of the multidimensional diversity of different microbial groups in Qinghai-Tibetan alpine wetlands and provide additional insights into how different microbial groups may respond to climate change.

Limitations of the study

In the present study, the microbial functional groups involved in soil nitrogen and carbon cycling we only measured nitrifiers, denitrifiers, methanogens, and methanotrophs, and further investigations will be needed in terms of more microbial functional groups involved in nitrogen and carbon cycling (such as carbon monoxide-oxidizing bacteria and anammox bacteria). Also, further studies should pay attention to the dynamic changes of biodiversity over time in the fields. In addition, this study was only conducted in the Qinghai-Tibetan Plateau wetlands; it is needed to investigate the ecological effects of microbial functional groups involved in nitrogen and carbon cycling in the world.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- METHOD DETAILS
  - Study sites
  - Soil sampling

- Plant diversity and abundance analyses
- Measurements of soil microbial diversity and abundance
- Measurement of environmental factors

● QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107252.

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AUTHOR CONTRIBUTIONS

B.D., G.L., and W.L., conceived the ideas and designed methodology; B.D., L.F., X.J., and S.B. collected the data; B.D., L.F., S.B., and W.L. analyzed the data; B.D., L.F., and W.L. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
MoBio PowerLyzer PowerSoil DNA isolation kit	Mo Bio	Cat. 12855
Deposited data		
Amplicon data of the bacterial 16S rRNA gene	This paper	NCBI Sequence Read Archive database: PRJNA657692
Amplicon data of the fungal internal transcribed spacer region of the ribosomal RNA gene	This paper	NCBI Sequence Read Archive database: PRJNA657715
Amplicon data of *nirK* and *nirS* genes	This paper	NCBI Sequence Read Archive database: SRP123686
Amplicon data of *amoA* gene	This paper	MG574722-MG574819
Amplicon data of *arch-amoA* gene	This paper	MG574595-MG574721
Amplicon data of *mcrA* gene	This paper	MH716827-MH717045
Amplicon data of *pmoA* gene	This paper	MH638914-MH638993
Oligonucleotides		
338F	Novogene Bioinformatics Technology Co., Ltd	ACTCCTACGGGAGGCAGCAG
806R	Novogene Bioinformatics Technology Co., Ltd	GGACTACHVGGGTWTCTAAT
ITS1	Novogene Bioinformatics Technology Co., Ltd	CTTGGTCATTTAGAGGAAGTAA
ITS2	Novogene Bioinformatics Technology Co., Ltd	GCTGCGTTCTTCATCGATGC
amoA-1F	Sangon Biotech Co., Ltd	GGGGTTTCTACTGGTGGT
amoA-2R	Sangon Biotech Co., Ltd	CCCCTCGGGAAAGCCTTCTTC
archea-amoAF	Sangon Biotech Co., Ltd	STAATGGTCTGGCTTAGACG
archea-amoAR	Sangon Biotech Co., Ltd	GCGGCCATCCATCTGTATGT
Cd3aF	Majorbio Bio-Pharm Technology Co. Ltd.	GTSAACGTSAAGGARACSGG
R3cd	Majorbio Bio-Pharm Technology Co. Ltd.	GASTTCGGRTGSGTCTTGA
F1aCu	Majorbio Bio-Pharm Technology Co. Ltd.	ATCATGGTSCTGCCGCG
R3Cu	Majorbio Bio-Pharm Technology Co. Ltd.	GCCTCGATCAG(A/G)TTGTGGTT
MLf	Sangon Biotech Co., Ltd	GGTGGTGTMGGATTCACACAR TAYGCWACAGC
MLr	Sangon Biotech Co., Ltd	TTCATTGCRTAGTTWGGRTAGTT
A189f	Sangon Biotech Co., Ltd	GGNGACTGGGACTTCTGG
Mb661r	Sangon Biotech Co., Ltd	CCGGMGCAACGTCYTTACC
Software and algorithms		
R 4.0.3	https://www.r-project.org/	https://www.r-project.org/
IBM SPSS Statistics 17	https://www.ibm.com/products/spss-statistics	https://www.ibm.com/products/spss-statistics
AMOS 20.0	https://sourceforge.net/projects/amos/	https://sourceforge.net/projects/amos/
QIIME version 1.17	https://qiime2.org	https://qiime2.org
Mothur version 1.23.0	https://mothur.org/wiki/download_mothur/	https://mothur.org/wiki/download_mothur/
ArcGIS 10.0	https://www.arcgis.com/index.html	https://www.arcgis.com/index.html
Other		
Illumina sequencing	Illumina	MiSeq

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Wenzhi Liu (liuwz@wbgcas.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data reported in this paper will be shared by the lead contact upon reasonable request.
- This study did not generate original code.
- Any additional information required to reanalyze the data reported in this work is available from the lead contact upon reasonable request.
- The authors declare that all data supporting the findings of this study are available within the Article and its Supporting Information files or from the corresponding authors upon reasonable request. The sequencing data using Illumina HiSeq 2000 and ABI-3730XL were submitted to the NCBI Sequence Read Archive database (accession numbers: PRJNA657692 for 16S rRNA, PRJNA657715 for ITS, SRP123686 for *nirK* and *nirS* genes, MG574722-MG574819 for *amoA*, MG574595-MG574721 for *arch-amoA*, MH716827-MH717045 for *mcrA*, and MH638914-MH638993 for *pmoA*).

METHOD DETAILS

Study sites

The Qinghai-Tibetan Plateau is the highest altitude and one of the largest (2.5 million km^2) plateaus on Earth. It holds 131894 km^2 of wetlands, accounting for approximately 27% of wetlands in China.[46] These wetlands are dominated by rivers, lakes, marshes, and artificial wetlands, such as reservoirs, paddy fields, and aquaculture ponds.[24] The Qinghai-Tibetan Plateau is known as "the water tower of Asia" because several major rivers in Asia have their source inside Tibet, such as the Yangtze, Yellow, Mekong, Salween, and Brahmaputra Rivers, and its downstream influence on approximately 40% of the global population.[24] Thus, the wetlands on the Qinghai-Tibetan Plateau play a significant role in providing water resources, improving water quality, and regulating hydrological cycles.[47]

The climate of the Qinghai-Tibetan Plateau is characterized by humid and wet summers and dry and cool winters, with a mean monthly temperature ranging between -9.4°C in January and 12.3°C in July. In most plateau areas, the annual mean precipitation is less than 400 mm, and over 80% of the total rainfall is concentrated in the summer monsoon season from June to August. Due to the extremely cold conditions, the surface soils of plateau wetlands start to freeze at the end of October and begin to thaw at the beginning of May. Due to the diverse range of climatic zones, highly varied topography, and long-term environmental stability, the Qinghai-Tibetan Plateau is recognized as one of the hotspots for a broad range of organisms.[48] In addition, two global biodiversity hotspots (the Hengduan and Himalaya Mountains) in the plateau are the potential origin pools of many temperate species.

Soil sampling

In July 2014, when surface soils had thawed, 7 palustrine, 9 riverine, and 20 lacustrine (lake margin) wetland sites on the Qinghai-Tibetan Plateau were non-randomly selected for collecting samples (Figures S1 and S2). These wetlands spanned a total distance of 1034 km north to south and 2457 km east to west and varied greatly in climate conditions, trophic status, and hydrological regimes (Table S1). At each wetland site, three plots (1 × 1 m) with a distance of at least 20 m between plots were established randomly to investigate plant diversity and abundance. Five soil cores (10 cm deep, 3 cm diameter) were collected from each plot and combined to represent the heterogeneity within this plot. After passing through a 1-cm sieve to remove the plant residues, root fragments, and gravel, soil samples were homogenized and divided into two parts. One part was placed in a centrifuge tube and frozen in liquid nitrogen for microorganism DNA extraction, and the other part was stored at approximately 5°C in a portable refrigerator to determine the soil physicochemical properties.

Plant diversity and abundance analyses

For each 1 m^2 plot, the vascular plant species and plant abundance were recorded. Plant species richness was defined as the number of vascular plant species in a plot.[49] The total plant cover in each plot was estimated visually using a 1×1 m grid frame that was divided into 0.1×0.1 m squares. Then, we determined the plant abundance of each plot according to a modified Braun-Blanquet cover system with six classes (1: less than 1%; 2: from 1 to 5%; 3: from 5 to 25%; 4: from 25 to 50%; 5: from 50 to 75%; 6: up to 75%).[50]

To compute plant phylogenetic diversity, we constructed a phylogeny including all vascular species in the 108 plots based on a published phylogenetic tree of vascular plants.[51,52] Our phylogenetic tree was created using the Phylomatic function,[53] and species missing from the published phylogenetic tree were placed in the tree at the crowns of their respective genera. Phylogenetic diversity, the sum of all phylogenetic branch lengths combining all species in a plot, was computed using the Phylocom 4.2 software.[54]

Measurements of soil microbial diversity and abundance

We only measured the soil microorganisms for the first plot in each wetland site because of limited funds. Microbial DNA was extracted from triplicate 0.25 g soils of each sample using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, USA) according to the manufacturer's instructions. The extracted DNA was detected by 1% agarose gel electrophoresis and quantified with a NanoDrop 2000 Fluorospectrometer (Thermo Fisher Scientific, Waltham, USA).

For soil bacteria and fungi, the V3-V4 region of the bacterial 16S rRNA gene and the fungal internal transcribed spacer (ITS) region of the ribosomal RNA gene were amplified by PCR using the primer pairs listed in Table S2. For microorganisms involved in N (ammonia-oxidizing bacteria, AOB; ammonia-oxidizing archaea, AOA; denitrifiers) and C (methanogens and methanotrophs) cycling, six functional genes (*amoA*, *arch-amoA*, *nirK*, *nirS*, *mcrA*, and *pmoA*) coding for key enzymes in the nitrification, denitrification, and methane production and oxidation pathways were also amplified by PCR using the primer pairs listed in Table S2. Each sample was subjected to triplicate amplification in a 20 μL reaction using the amplification conditions listed in Table S2.[43,55] The PCR products from each sample were pooled and purified by gel electrophoresis and extracted using an AxyPrep DNA Gel Extraction Kit (Axygen).

For the 16S rRNA, ITS, *nirK*, and *nirS* genes, the purified PCR products were paired-end sequenced using an Illumina HiSeq 2000 sequencer (Illumina, San Diego, USA). The sequencing data were submitted to the NCBI Sequence Read Archive database (accession numbers: PRJNA657692 for 16S rRNA, PRJNA657715 for ITS, and SRP123686 for *nirK* and *nirS* genes). The raw sequences were merged using FLASH and then were quality-filtered using QIIME version 1.17. For the *amoA*, *arch-amoA*, *mcrA*, and *pmoA* genes, the purified PCR products were cloned into a pMD18-T vector (TaKaRa, Dalian, China) and then transformed into Trans-5α competent cells (Transgen Biotec, Beijing, China). Sixty positive clones were sequenced with an ABI-3730XL (Applied Biosystems, CA, USA). All represented sequences were deposited in GenBank with the following accession numbers: MG574722-MG574819 for *amoA*, MG574595-MG574721 for *arch-amoA*, MH716827-MH717045 for *mcrA*, and MH638914-MH638993 for *pmoA*. The sequences were assigned to operational taxonomic units (OTUs) at 97% (for 16S rRNA and ITS) or 95% (for *amoA*, *arch-amoA*, *nirK*, *nirS*, *mcrA*, and *pmoA*) similarity level using the Mothur program by the furthest neighbor algorithm.

The species richness of soil microbes was evaluated using the Chao1 richness estimator in the Mothur software program (Version 1.23.0). Phylogenetic diversity was measured based on Faith's approach, which is the sum of the total phylogenetic branch length of detected OTUs in each sample. To calculate the phylogenetic diversity of soil microbes, phylogenetic trees were generated for 16S, ITS, *amoA*, *arch-amoA*, *nirK*, *nirS*, *mcrA*, and *pmoA* data sets, respectively.[34] The OTU representative sequences for 16S, ITS, *amoA*, *arch-amoA*, *nirK*, *nirS*, *mcrA*, and *pmoA* sequences were aligned using Mothur software (Version 1.23.0). Subsequently, the phylogenetic trees were constructed, and Faith's phylogenetic diversity values were calculated in QIIME.

The abundance (i.e., copy number) of the above eight genes (16S rRNA, ITS, *amoA*, *arch-amoA*, *nirK*, *nirS*, *mcrA*, and *pmoA*) was measured in triplicate by using a Roche LightCycler 480 real-time PCR system (Roche Diagnostics, Mannheim, Germany) with the fluorescent dye SYBR green quantitative PCR method.[47] The primer sets of 338F/806R, ITS1/ITS2, amoA-1F/amoA-2R, Arch-amoAF/ArchamoAR, nirSCd3aF/nirSR3cd,

nirK876/nirK1040, mlas/mcrA-r, and A189f/mb661r were used for 16S rRNA, ITS, *amoA*, *arch-amoA*, *nirS*, *nirK*, *mcrA*, and *pmoA* genes, respectively. Each 20 μL quantitative qPCR reaction contained 10 μL of SybrGreen qPCR Master Mix (2 ×), 1 μL of primers (10 μM), and 2 μL of DNA template. Standard curves were created by using serial plasmid dilutions of known amounts of plasmid DNA-containing standard samples.

Measurement of environmental factors

At each sampling site, the elevation, longitude (°E), and latitude (°N) were recorded using a global positioning system (Unistrong Co., Ltd, Beijing, China). The climatic factors, including mean annual precipitation (MAP) and mean annual temperature (MAT), were extracted from a national climate data set at a spatial resolution of 1 km in ArcGIS 10.0 (ESRI Inc., Redlands, CA, USA).

Field soil temperature (ST) was measured by inserting a stainless temperature probe into the 5-cm soil layer of each plot. In the laboratory, soil pH and conductivity (EC) were determined with a pH/conductivity electrode at a soil to water ratio of 1:5 (v/v). Wet basis moisture was assessed gravimetrically after drying 50 g soil samples at 105°C for 24 hours in an oven. Soil ammonia (NH_4^+) and nitrate (NO_3^-) concentrations were determined by extracting 10 g of fresh soil with 100 mL of 2 mol/L KCl solution and then using an automatic nutrient analyzer (EasyChem plus, Systea, Italy). After air-drying and sieving, soil samples were analyzed for total carbon (TC) and total nitrogen (TN) concentrations using an elemental analyzer (Vario TOC cube, Hanau, Germany). The concentration of soil total phosphorus (TP) was measured by the molybdenum blue method with a spectrophotometer (Shimadzu UV-1800, Tokyo, Japan) after digestion.

QUANTIFICATION AND STATISTICAL ANALYSIS

The data were analyzed with the Shapiro–Wilk test for normality and were logarithm or square root transformed to improve normality when appropriate. The differences in species and phylogenetic diversity among the three wetland types were compared using one-way ANOVA with Duncun's post hoc test. The relationship between biodiversity and all measured variables (elevation, climatic factors, and soil attributes) was assessed using Pearson correlation coefficients. The correlation between species abundance and environmental parameters was performed using R software version 4.0.3 (R Project for Statistical Computing). The goodness of fit between biodiversity and elevation was estimated with polynomial regression. For the polynomial regression, R^2 was calculated to make a comparison between the first-order and the second-order polynomial models.[56] Statistical analyses were performed using SPSS software version 17.0 (IBM Inc., Chicago, IL, USA).

Structural equation modeling (SEM) was used to assess the direct and indirect effects of each environmental variable on biodiversity. SEM was conducted using AMOS 20.0 (Amos Development Corporation, Chicago, IL, USA). The prior model (Figure S3) was constructed according to the known effects and the relationships between biodiversity and soil physiochemical characteristics.[57] Elevation, wetland type, MAT, and MAP were also included in our models, given their effects on biodiversity and on the rest of the variables evaluated. We then modified our models according to the relationship ($P < 0.05$) between soil attributes and biodiversity. All final SEMs had a high comparative fit index (CFI >0.90) and a goodness of fit index (GFI >0.90).