

# Increasing soil pH enhances the network interactions among bacterial and archaeal microbiota in alpine grasslands of the Tibetan Plateau

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## Research

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# Abstract

**Background:** Soil functioning and processes are driven by complex microbial interactions. It is therefore critical to understand the co-occurrence patterns of soil microbiota, especially in fragile alpine ecosystems. Here we explored the geographic patterns of the topological features and the major drivers shaping the topological structure of the co-occurrence network for bacterial and archaeal microbiota in alpine grasslands of the Tibetan Plateau based on high-throughput sequencing.

**Results:** Soil pH was the most important environmental variable for predicting the topological features of the microbial network at both network and node levels. Associations among soil microbiota were enhanced with increasing pH (5.17–8.92), and the network was the most stable at neutral pH (7). Node-level topological features suggested that taxa of the high-pH cluster had more important roles in maintaining complex network connections than taxa of the low-pH cluster. Network-level features revealed closer relationships among soil microbiota in the steppe ecosystem than in the meadow ecosystem. Archaeal operational taxonomic units (OTUs) with higher values for node-level topological features appeared to be more important than bacterial OTUs in maintaining complex connections. The co-occurrence patterns of bacterial OTUs with lower node-level feature values followed a power-law distribution, whereas those of archaeal OTUs did not.

**Conclusions:** Soil pH plays a decisive role in determining the complex interactions among soil microbiota in alpine grassland ecosystems of the Tibetan Plateau, with a more stable co-occurrence network at neutral pH. Bacterial and archaeal taxa, which occupy distinct network niches, have closer relationships in alpine steppe than in alpine meadow ecosystems.

## Background

Soil microorganisms play an indispensable role in mediating the biogeochemical cycles of nutrient elements such as C, N, S, and P, and in maintaining ecosystem functions and services [1–3]. A growing body of evidence shows that soil microbial communities can shape and be shaped by plant traits and soil properties. Thus, microorganisms are key ecological linkages between aboveground and belowground communities [4, 5]. Most importantly, microorganisms in a complex soil matrix generally interact with each other to resist the external environment, such as in the form of prey–predator and parasite–host interactions [6]. Microbial interactions drive the ecological selection process governing microbial community assembly [7], and therefore make a contribution to biogeochemical cycles and ecosystem services [8, 9]. Potential interactions among soil microbiota have been well demonstrated in a broad range of ecosystem types and among various habitat types based on co-occurrence network analysis [10–12].

The Tibetan Plateau, the highest and largest plateau on the Earth [13, 14], is highly sensitive to global change and human activities [15]. Importantly, alpine grassland ecosystems make a substantial contribution to climate regulation, water conservation, and soil formation across the Tibetan Plateau [16]. Due to global warming and vegetation degradation, soil environmental and ecosystem processes in the

Tibetan Plateau region are undergoing tremendous changes [17–19]. Furthermore, compared with other ecosystems, alpine grasslands have a higher abundance of herbaceous litter [20, 21] and store a larger amount of soil organic carbon under low temperature conditions [22]. Given the unique characteristics of alpine ecosystems, the microbially mediated soil processes and ecological diversity patterns on the Tibetan Plateau should be distinct to those observed in other natural and agricultural ecosystems. Understanding the co-occurrence patterns of soil microbiota in alpine ecosystems will help to predict soil microbial interactions in response to global change. However, this is poorly understood in the case of the Tibetan Plateau.

Exploring the geographic patterns of the co-occurrence network topological features for soil microbiota can provide insights into the assembly of microbial communities on large spatial scales [11]. Bacterial, rather than archaeal, networks become more complex as precipitation increases in semi-arid grassland soils [23]. Soil pH plays an essential role in the interactions within diazotrophic communities in the rhizosphere and bulk soils of wheat fields [24]. Abundant bacteria show closer relationships to, and a greater influence on, other co-occurring taxa in the community than rare bacteria in oil-contaminated soils [25]. However, these studies have focused on soil bacterial, fungal, or archaeal communities in non-alpine ecosystems separately [12]. Currently, little is known about the interactions among soil bacterial and archaeal taxa in alpine ecosystems, especially across large spatial scales. In addition, our knowledge of the dominant drivers of soil microbial networks lags behind that reported for aboveground biotic interactions [26]. Therefore, a predictive understanding of factors affecting the geographic patterns of co-occurrence network topological features for soil microbiota is urgently needed.

Ecological clusters, which reveal niche spaces shared by microbial community members [10], can be used to identify the environmental preferences of highly connected taxa by integrating high-dimensional data into predictable groups [27, 28]. The grouping of soil microbial taxa into various ecological clusters is expected to have multiple implications for the maintenance of soil fertility, decomposition processes, and plant productivity in terrestrial environments [29–31]. A recent study has suggested that archaeal taxa tend to co-occur and form well-defined ecological clusters of exclusive taxa in agricultural soils across eastern China [32]. However, the spatial distributions of ecological clusters of soil microbial taxa have not been well investigated in the Tibetan alpine grasslands. Additionally, it remains unclear which ecological clusters (formed by integrating bacterial and archaeal taxa) play a key role in the co-occurrence network for soil microbiota in alpine ecosystems.

Here, a comprehensive study was conducted on a regional scale by intensively selecting 62 sites arrayed along extensive climatic, edaphic, and floristic gradients in alpine meadow and steppe ecosystems of the Tibetan Plateau. The objective of this study was to explore the geographic patterns of the co-occurrence network topological features for soil bacterial and archaeal microbiota in alpine grassland ecosystems without human activities. The co-occurrence patterns in natural alpine grasslands could be used to help ascertain reliable the geographic patterns of soil microbiota by eliminating anthropogenic influences. Specifically, the dominant drivers of the network topological structure were determined across the alpine grasslands, and the topological features of the nodes associated with different ecological clusters were

compared. To solve these problems, a high-throughput sequencing and network-based analysis was performed using integrated bacterial and archaeal datasets. It was hypothesized that: (i) the topological features of the co-occurrence network for soil bacterial and archaeal microbiota exhibit distinct geographic patterns across the alpine grasslands; (ii) soil pH plays a key role in determining soil microbial interactions at both network and node levels; and (iii) dominant bacterial and archaeal taxa share similar ecological clusters in alpine ecosystems of the Tibetan Plateau. The results of this study could provide fundamental insight into how the interactions among soil microbiota respond to global environmental change in alpine ecosystems.

## Results

### Datasets

A total of 2,882,628 and 4,264,236 high-quality bacterial and archaeal sequences, respectively, were obtained from 186 soil samples collected in natural alpine grasslands of the Tibetan Plateau (Additional file 2: Fig S1). The data set was subsampled to 15498 and 22926 reads for bacteria and archaea per sample, respectively. The sequences were clustered into 10,624 bacterial operational taxonomic units (OTUs) and 9,944 archaeal OTUs based on 97% sequence similarity. The majority of bacterial sequences were from the phyla (and sub-phyla) Actinobacteria (32.4%), Alphaproteobacteria (16.9%), Acidobacteria (14.0%), Betaproteobacteria (4.3%), Gemmatimonadetes (3.2%), and Deltaproteobacteria (3.1%). The archaeal sequences mainly belonged to the phyla Thaumarchaeota (87.7%) and Euryarchaeota (3.8%).

Here, the OTUs ranking the top 20% in their relative abundance and occurring in more than half of all soil samples were defined as dominant taxa for bacteria and archaea. In total, 1276 bacterial and 259 archaeal dominant taxa were identified, which accounted for 12.0% of total bacterial and 2.6% of total archaeal taxa observed. Nevertheless, on average, the dominant taxa accounted for 75.4% and 82.7% of total sequences for bacteria and archaea, respectively.

#### Co-occurrence patterns of dominant bacterial and archaeal taxa

A meta-community co-occurrence network was established based on the Spearman's correlations among the dominant bacterial and archaeal taxa. The network contained 1105 nodes (OTUs) and 68,679 edges (Fig. 1A). Of these nodes, 850 and 255 belonged to bacterial and archaeal taxa, respectively. There were more edges between the archaeal and bacterial nodes (31.1%) than between the archaeal nodes (6.2%). Interestingly, the values for the node-level topological features including betweenness centrality, eigenvector centrality, and closeness centrality were significantly higher ( $p < 0.001$ ) in archaeal OTUs than in bacterial OTUs (Fig. 1B).

The meta-community co-occurrence network roughly followed a scale-free degree distribution (Additional file 2: Fig S2), indicating a scale-free network structure and non-random co-occurrence pattern. Additionally, the degrees for bacterial interactions followed a power-law distribution, whereas the distribution of archaeal OTUs did not (Additional file 2: Fig S3).

## Distinct network-level topological features in alpine grassland soils

A set of network-level topological features were calculated for each soil sample by maintaining OTUs associated with specific samples in the meta-community co-occurrence network (Additional file 1: Table S1). The network-level topological features could be divided into three clusters based on correlation and hierarchical cluster analyses of the dissimilarities (Additional file 1: Fig S4). The first cluster included average path length, betweenness centralization, modularity, and eigenvector centralization. The second cluster contained degree assortativity and diameter. The third cluster included edge number, vertex number, average degree, density, clustering coefficient, degree centralization, and closeness centralization. The first cluster of network-level topological features was negatively correlated with soil pH, whereas the second and third clusters were positively correlated with soil pH. In contrast, other environmental factors were positively correlated with the first cluster, and positively correlated with the second and third clusters.

Across different habitats, soil pH was significantly higher in the alpine steppe than in the alpine meadow. However, soil moisture (SM), soil organic matter (SOM), total phosphorus (TP), available phosphorus (AP), available nitrogen (AN;  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), and mean annual precipitation (MAP) were higher in the alpine meadow than in the alpine steppe (Additional file 1: Fig S5). Similar to soil pH, values for the edge number, average degree, density, clustering coefficient, degree centralization, and closeness centralization were higher for sub-networks in the alpine steppe than for those in the alpine meadow according to Wilcoxon rank sum tests (Fig. 2). In contrast, values for the average path length, betweenness centralization, eigenvector centralization, and modularity were lower for sub-networks in the alpine steppe than for those in the alpine meadow.

## Network-level topological features driven by environmental filtering

To disentangle the effects of environmental and geographic factors on network-level topological features, standard and partial Mantel tests (Spearman's correlation) were carried out on the environmental (Euclidean) and geographical distances. Geographical distance was generated from the longitude and latitude coordinates of each sampling site. Compared with geographic factors, the environmental factors contributed to more of the variation in the network-level topological features of the microbial network (Tables 1 and 2). A random forest analysis was then applied to identify the major environmental factors contributing to the variation in network-level topological features. Soil pH appeared to be the primary factor affecting the edge number, average path length, average degree, vertex number, density, degree centralization, eigenvector centralization, and modularity of the microbial network (Fig. 3).

Table 1

Partial Mantel test results for the correlation between network-level topological features and environmental variables in the Tibetan alpine grasslands

Partial Mantel statistic r (P value) for <sup>b</sup> :	
Variable <sup>a</sup>	Network-level topological features
pH	0.66 (0.001 <sup>***</sup> )
SM %	0.16 (0.001 <sup>***</sup> )
SOM %	0.21 (0.001 <sup>***</sup> )
TN (mg/g)	0.058 (0.02 <sup>*</sup> )
TP (mg/g)	0.24 (0.001 <sup>***</sup> )
AP (mg/kg)	0.073 (0.004 <sup>**</sup> )
NO <sub>3</sub> <sup>-</sup> -N (mg/kg)	0.084 (0.002 <sup>**</sup> )
NH <sub>4</sub> <sup>+</sup> -N (mg/kg)	0.091 (0.001 <sup>***</sup> )
MAT (°C)	0.059 (0.004 <sup>**</sup> )
MAP (mm)	0.32 (0.001 <sup>***</sup> )
<sup>a</sup> pH, soil pH; SOM, soil organic matter; AP, soil available phosphorus; TN, total nitrogen; TP, total phosphorus; SM, soil moisture content; NO <sub>3</sub> <sup>-</sup> -N, nitrate-nitrogen; and NH <sub>4</sub> <sup>+</sup> -N, ammonium-nitrogen; MAT, mean annual temperature; and MAP, mean annual precipitation	
<sup>b</sup> Statistical significance was tested based on 999 permutations. Asterisks represent significance of correlation (*P < 0.05, **P < 0.01, and ***P < 0.001).	

Table 2

Mantel and partial Mantel test results for the correlation between network-level topological features and environmental or geographical distance in the Tibetan alpine grasslands

		Mantel statistic r (P value) for <sup>a</sup> :
Effect of	Controlling for	Network-level topological features
Environmental distance		0.31 (0.001 <sup>***</sup> )
Geographical distance		0.075 (0.001 <sup>***</sup> )
Environmental distance	Geographical distance	0.31 (0.001 <sup>***</sup> )
Geographical distance	Environmental distance	-0.075 (n.s.)

<sup>a</sup>Statistical significance was tested based on 999 permutations. Asterisks represent significance of correlation (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001; and n.s., P > 0.05).

These observations were supported by the results of multivariate regression analysis (Additional file 1: Table S2). Interestingly, significant and positive linear regressions were found between soil pH and the edge number, average degree, vertex number, density, and degree centralization. In contrast, significant and negative linear regressions were found between soil pH and the average path length, eigenvector centralization, and modularity (Fig. 4; Additional file 2: Fig S6). Additionally, there were significantly positive relationships between soil pH and the number of positive edges, both within kingdoms and between kingdoms (Additional file 2: Fig S7). Moreover, the number of negative edges peaked at neutral pH, both within kingdoms and between kingdoms (Additional file 2: Fig S7).

#### Spatial atlases of network-level topological features for soil microbiota

To predict the spatial distribution of network-level topological features for the microbial network, kriging interpolation was performed on each network-level topological feature. The predicted spatial patterns showed that the edge number, average degree, and vertex number were higher in high-pH soil (alpine steppe) than in low-pH soil (alpine meadow; Fig. 4). Similar patterns were observed for the density, degree centralization, and positive edge number within or between kingdoms (Additional file 2: Fig S6). In contrast, the average path length of sub-networks for soils was lower in high-pH soil than in low-pH soil (Fig. 4). The distributions of betweenness centralization, eigenvector centralization, and modularity were similar to patterns observed for the average path length (Additional file 2: Fig S6).

#### Ecological clusters of dominant bacterial and archaeal communities

To explore the effects of environmental and geographic factors on soil microbial community structure, standard and partial Mantel tests (Spearman's correlation) were applied to distinguish significant factors. Compared with geographic factors, the environmental factors played more important roles in shaping both the bacterial and archaeal community structures (Tables 3 and 4). The constrained analysis of

principal coordinates (CAP) revealed that both dominant bacterial and archaeal communities were primarily affected by soil pH (Fig. 5A and B; Additional file 1: Tables S3 and S4). The ecological preferences of dominant bacterial and archaeal taxa were then determined by focusing on soil pH based on Spearman correlations ( $P < 0.05$ ). The dominant bacterial and archaeal taxa were clustered into two ecological clusters sharing habitat preferences for (i) high pH and (ii) low pH (Additional file 1: Table S5).

Table 3

Partial Mantel test results for the the correlation between dominant microbial community similarity and environmental variables in the Tibetan alpine grasslands

Variable	Partial Mantel statistic r (P value) for <sup>b</sup> :	
	Bacteria	Archaea
pH	0.70 (0.001 <sup>***</sup> )	0.64 (0.001 <sup>***</sup> )
SM %	0.28 (0.001 <sup>***</sup> )	0.16 (0.001 <sup>***</sup> )
SOM %	0.40(0.001 <sup>***</sup> )	0.21 (0.001 <sup>***</sup> )
TN (mg/g)	0.18(0.001 <sup>***</sup> )	0.073 (0.01 <sup>**</sup> )
TP (mg/g)	0.26 (0.001 <sup>***</sup> )	0.20 (0.001 <sup>***</sup> )
AP (mg/kg)	0.13 (0.001 <sup>***</sup> )	0.090 (0.001 <sup>***</sup> )
NO <sub>3</sub> <sup>-</sup> -N (mg/kg)	0.059 (0.005 <sup>**</sup> )	0.090 (0.002 <sup>**</sup> )
NH <sub>4</sub> <sup>+</sup> -N (mg/kg)	0.084 (0.001 <sup>***</sup> )	0.066 (0.007 <sup>**</sup> )
MAT(°C)	0.21 (0.001 <sup>***</sup> )	0.12 (0.001 <sup>***</sup> )
MAP(mm)	0.36 (0.001 <sup>***</sup> )	0.31 (0.001 <sup>***</sup> )
<sup>a</sup> pH, soil pH; SOM, soil organic matter; AP, soil available phosphorus; TN, total nitrogen; TP, total phosphorus; SM, soil moisture content; NO <sub>3</sub> <sup>-</sup> -N, nitrate-nitrogen; and NH <sub>4</sub> <sup>+</sup> -N, ammonium-nitrogen; MAT, mean annual temperature; and MAP, mean annual precipitation		
<sup>b</sup> Statistical significance was tested based on 999 permutations. Asterisks represent significance of correlation (*P < 0.05, **P < 0.01, and ***P < 0.001).		



Table 4

Mantel and partial Mantel test results for the correlation between dominant microbial community similarity and environmental or geographical distance in the Tibetan alpine grasslands

		Mantel statistic r (P value) for <sup>a</sup> :	
Effect of	Controlling for	Bacteria	Archaea
Environmental distance		0.41 (0.001 <sup>***</sup> )	0.34 (0.001 <sup>***</sup> )
Geographical distance		0.19 (0.001 <sup>***</sup> )	0.13 (0.001 <sup>***</sup> )
Environmental distance	Geographical distance	0.37 (0.001 <sup>***</sup> )	0.32 (0.001 <sup>***</sup> )
Geographical distance	Environmental distance	0.013 (n.s.)	-0.028 (n.s.)

<sup>a</sup>Statistical significance was tested based on 999 permutations. Asterisks represent significance of correlation (\*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001; and n.s., P > 0.05).

The significant relationships between soil pH and the relative abundance of specific ecological clusters indicated that the clusters were reasonably well defined by soil pH (Fig. 5C and D). Spatial distribution maps of the dominant bacterial and archaeal taxa were constructed using the kriging process to interpolate the relative abundance of each ecological cluster in alpine grasslands (Fig. 5E and F). The maps provided estimates of the areas where the clusters of the dominant bacterial and archaeal taxa would be expected to be most abundant. For instance, the low-pH and high-pH clusters were relatively abundant in low-pH and high-pH soils, respectively. Each of the ecological clusters contained multiple genera of soil microbiota. *Rubrobacter*, *Blastococcus*, *Sphingomonas*, *Microvirga*, *Solirubrobacter*, *Skermanella*, *Crossiella*, and *Nitrososphaera* preferred high soil pH, whereas *Bradyrhizobium*, *Nitrospira*, *Bacillus*, *Pseudonocardia*, *Mycobacterium*, *Defluviicoccus*, and *Solibacter* preferred low soil pH. *RB41*, *Gaiella*, and *Roseiflexus* were relatively abundant in both high-pH and low-pH soils (Additional file 2: Fig S8).

Correlation network analyses confirmed that the dominant bacterial and archaeal taxa that shared similar habitat preferences tended to co-occur (Fig. 1A). Interestingly, values for the node-level topological features including the degree, betweenness centrality, eigenvector centrality, and closeness centrality were significantly higher ( $P < 0.01$ ) in the high-pH cluster than in the low-pH cluster (Fig. 2B). This indicated that taxa of the high-pH cluster played major ecological roles in maintaining complex connections with other taxa. Sub-networks were generated for bacteria and archaea, respectively, which showed that taxa of the high-pH cluster had higher node-level topological values than taxa of the low-pH cluster (Additional file 2: Fig S9). Additionally, the degrees of bacterial sub-networks for high-pH and low-pH clusters followed a power-law distribution, whereas the archaeal sub-networks did not (Additional file 2: Fig S3).

## Discussion

By accounting for the complex interactions among bacterial and archaeal taxa, we explored the geographic patterns of the topological features of the co-occurrence network for soil microbiota in the alpine grasslands of the Tibetan Plateau. In this region, cold, high ultraviolet radiation, low oxygen [22], and vegetation degradation [17] result in different metabolism strategies, assembly processes, and biogeographical patterns of soil microbiota compared to those within other soil ecosystems. The results of this study supported the proposed hypothesis that the geographic patterns of the overall network features for soil bacterial and archaeal microbiota varied distinctly in alpine grasslands across the environmental gradients on a regional scale. The influence of soil pH on soil microbial interactions overwhelmed that of the environmental other factors. Different kingdoms occupied the same habitat niche due to their similar environmental preferences. These results highlight the ecological role of soil microbiota in regulating ecosystem structure, processes, and functioning in alpine ecosystems.

Network topological features could reflect the co-occurrence patterns of soil microbiota [11]. For example, nodes with higher node-level topological feature values play central roles in the network, whereas lower topological feature values indicate a peripheral position [11, 25, 33]. Network-level topological features with a high value (such as average degree, clustering coefficient, and density) indicate a more connected network, whereas those with lower values (such as average path length and diameter) suggest closer connections within the network [10, 11, 33]. Here, closer relationships and similar environmental preferences were observed between soil archaeal and bacterial taxa in the alpine grasslands. These observations could be expected as soil microorganisms interact with each other to resist changes in external environments [6]. These results confirmed that soil microorganisms could have commonalities with regards to their response to ecological selection; they prefer the same substrates and environmental properties [34, 35]. In contrast to what was observed for the bacterial taxa, microbial interactions were stronger between kingdoms than within kingdoms for archaeal taxa. This discrepancy indicated that archaea could strengthen the robustness of the co-occurrence network for soil microbiota in the Tibetan alpine grasslands [36]. We supposed that archaea and bacteria had complementary physiologies (for example, as is seen with the copolymer of ammonia-oxidizing archaea and nitrite-oxidizing bacteria [37]) and participated in horizontal gene transfer.

In this study, archaeal taxa were more often located in core positions in the network than bacterial taxa. This phenomenon suggested that archaeal taxa played more major ecological roles in maintaining complex connections than bacterial taxa in the Tibetan alpine grasslands. The presence of Thaumarchaeota as a dominant archaeal phylum might be explained by three main factors. First, compared with ammonia-oxidizing bacteria, ammonia-oxidizing archaea have a higher affinity for substrate and oxygen; therefore, the latter group could survive under oligotrophic conditions during the biogeochemical cycles of carbon and nitrogen [38]. Second, unlike ammonia-oxidizing bacteria, ammonia-oxidizing archaea assimilate CO<sub>2</sub> via an energy-saving method (the 3-hydroxypropionate/4-hydroxybutyrate pathway) for autotrophic growth, and thereby use organic carbon sources directly [39, 40]. Third, in addition to autotrophic metabolism, ammonia-oxidizing archaea are capable of mixotrophic growth [41, 42] and develop a heterotrophic metabolism using organic matter to sustain cell growth on account of survival pressure caused by an energy shortage [43]. Euryarchaeota prefer anoxic

environments and grow autotrophically using a variety of metabolic pathways [44]. These unique physiological characteristics of archaeal taxa might allow them to deploy diverse metabolic functions, which would, in turn, enable them to adapt to the extreme environmental conditions of low temperature, low oxygen, and oligotrophic conditions found on the Tibetan Plateau.

In particular, significant relationships suggested that the soil pH determined the topological features of the co-occurrence network for soil microbiota in the Tibetan alpine grasslands. One possible explanation for this is that soil pH could affect soil microbial interactions via regulating the availability of soil nutrients [45, 46]. Based on the strong relationships between soil pH versus SOM, TN, AN ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), TP, and AP, soil pH was likely to influence the interactions among soil bacterial and archaeal taxa via changing the C, P, and N availability in the alpine grasslands (Additional file 2: Fig S10). Another explanation is that the pH range for optimal growth of bacterial communities is relatively narrow [47, 48], and soil pH is the dominant contemporary factor that shifts ammonia-oxidizing archaeal communities [49, 50]. Here we also found that the taxa of the high-pH cluster played more important ecological roles in sustaining complex connections in the co-occurrence network than taxa of the low-pH cluster. This may be due to the core taxa being dominant in the high-pH cluster, which may have been more connected than the other taxa [51]. Additionally, we observed stronger competition in the soil microbiota both between and within kingdoms in neutral soil pH conditions. This result indicated that the topological structure of the co-occurrence network for the soil microbiota was more stable in alpine grassland soils with a neutral pH. This is due to the fact that the interactions of soil microbial taxa, which are connected through a large proportion of positive edges, result in tandem responses to environmental changes, leading to positive feedback and co-oscillation. Meanwhile, an increasing number of negative edges improve the stability of the microbial network subjected to disturbances by stabilizing co-oscillation [52, 53].

Alpine grassland ecosystems have two dominant vegetation types: alpine steppe, which is characterized by low MAP, oligotrophic conditions, and a high soil pH, and alpine meadow, which is characterized by high MAP, copiotrophic conditions, and a low soil pH [54]. The results of the present study were somewhat in accordance with these characteristics of alpine steppe and meadow ecosystems (Additional file 2: Fig S5). A significant positive correlation was found between the soil pH and the edge number, vertex number, average degree, density, and degree centralization of the co-occurrence network for soil microbiota in the alpine grasslands. This indicated that the microbial associations tended to be stronger among OTUs that typified high soil pH than among those OTUs that typified low soil pH on the Tibetan Plateau. This tendency was supported by the spatial distribution maps of network topological features for soil microbiota. The maps showed that soil bacterial and archaeal taxa in the alpine steppe (high soil pH) had closer relationships than in the alpine meadow (low soil pH). One plausible explanation for this observation is that the bacterial and archaeal communities in N- and P-limited alpine grasslands [55, 56] are dominated by oligotrophic clades such as Actinobacteria, Acidobacteria, and ammonia-oxidizing archaea [38, 57]. Therefore, soil bacterial and archaea have stronger connections in the alpine steppe (oligotrophic conditions and high soil pH) to resist pressure caused by an energy deficit [30]. Another explanation is the serious degeneration of alpine grasslands as a result of high variation in soil sand

content between the alpine steppe (high sand content) and the alpine meadow (low sand content) ecosystems of the Tibetan Plateau [58]. The high sand content and water may have provided a better microenvironment for the interactions within soil microbial communities. In addition, the spatial patterns of relative abundance were constructed for each ecological cluster of abundant bacterial and archaeal taxa. These patterns suggested that taxa of the high-pH and low-pH clusters were relatively abundant in known areas of high and low soil pH, respectively. This is consistent with the tendency that node-level topological feature values for the high-pH cluster were higher than values for the low-pH cluster.

The results of the present study suggested that the degree distribution for edges between bacterial nodes followed a power-law distribution, whereas the distribution of archaeal interactions showed a random co-occurrence pattern. Many previous studies that have revealed that archaeal networks followed a random distribution in forest soils, and in flooded or non-flooded soils [11, 24, 59]. Herein, the random degree distribution pattern of soil archaea indicated that each connection between paired archaeal taxa was equal. This suggests an increased distributional homogenization for different species of archaea in the Tibetan alpine grassland soils compared with other soils. Our observation could be explained by the following factors: first, different degree distribution patterns for various soil microbial communities are related to their assembly processes, which has been observed in forest soils and wheat rhizosphere soils [11]; second, soil archaeal community has a stochastic assembly process, resulting in weak habitat dependence [60].

Bacterial and archaeal taxa have ecological coherence [61, 62], respectively, such as in terms of their physiological capabilities [57, 63]. Among the microbial taxa detected in the Tibetan alpine grasslands, acid-tolerant *Bradyrhizobium* species of the low-pH cluster had wide niches and preferred low-pH environments [64, 65]. In addition, *Rubrobacter* from the high-pH cluster, and *Mycobacterium* and *Candidatus Solibacter* of the low-pH cluster, were coincidentally observed in a previous study that defined the ecological clusters of high pH and low pH [66]. Moreover, *Sphingomonas* and *Skermanella* of the high-pH cluster were found in drylands [66]. This may be related to the fact that taxa of the high-pH cluster were dominant in the alpine steppe, which had a lower water content and soil organic matter content than the alpine meadow ecosystem [54]. Some interesting results were observed; for example, *Nitrososphaera* species preferred a high-pH soil environment, and this genus has been reported to be alkaliphilic [67]. These consistent lines of evidence improve the overall reliability of the environmental preferences predicted in this study for the dominant soil bacteria and archaea in the Tibetan alpine grasslands. Additionally, some genera found in the alpine soils, including *Sphingomonas*, *Bacillus*, *Nocardioides*, *Gaiella*, and *Bradyrhizobium*, also occurred in agro-soils [51], which indicates their universal distribution. Meanwhile, *RB41*, *Blastococcus*, *Microvirga*, *Roseiflexus*, *Crossiella*, *Nitrospira*, and *Defluviicoccus* were identified in the Tibetan alpine soils; these unique bacterial genera may serve as indicator groups for alpine ecosystems. These findings provide fundamental information for predicting the preferred environmental conditions of dominant bacterial and archaeal taxa and for enriching particular species in vitro, which could increase the probability of their successful cultivation.

## Conclusions

This study provides a complementary viewpoint to microbial ecology research by integrating the complex interactions among soil bacterial and archaeal microbiota into predictable topological features of the co-occurrence network. The results indicated that soil pH played a decisive role in driving the distinct geographic patterns of the topological structure of the co-occurrence network for soil microbiota in the alpine grasslands of the Tibetan Plateau. Interestingly, microbial taxa in the alpine steppe ecosystem were more closely associated than in the alpine meadow ecosystem. Archaeal and high-pH cluster had higher values for node-level topological features, suggesting that these taxa played core ecological roles within the network. The associations among soil microbiota were enhanced with increasing pH, and the microbial network was the most stable at neutral pH. By identifying the dominant bacterial and archaeal taxa along with their habitat preferences, the spatial distribution of relative abundance was determined for each cluster of the dominant microbial taxa. These findings improve our ability to predict soil microbial interactions in alpine ecosystems in response to global warming and anthropogenic disturbances. Future investigations ought to focus on the phylogenetic relationships between bacterial and archaeal taxa due to their similar environmental preferences and strong connections. This approach is also critical to better understand what driving factors affect the interactions among soil microbiota in the face of ongoing global environmental change.

## Methods

### Soil sampling

One hundred and eighty-six soil samples from sixty-two study sites were selected on the Tibetan Plateau during the peak vegetation growing season (July-August) in 2015-2016 years to target maximum microbial activity and biomass (Additional file 2: **Fig S1**). The sampling sites extend from 29.14 °N to 36.06 °N and 88.15 °E to 101.20 °E along the whole scale. The survey area mainly included two vegetation types (alpine meadow and alpine steppe), an annual average temperature between -15 °C and 5 °C [68] and about ~90% precipitations mainly occur during July and August ranging from 100 to 700 mm per annum on the Tibetan Plateau grassland [13, 69]. Dominant plant species are *Kobresia pygmaea* (Cyperaceae), *Tibet. pygmaea* (Cyperaceae), *Festuca ovina* (Poaceae) and *Poa poophagorum* (Poaceae). Within each plot from the top 0–15 cm of the soil column, Five-point samples located soil cores with a diameter of 3.8 cm were collected and mixed to obtain one composite sample. Every composite soil sample was sieved through a 2 mm mesh to remove plant debris and rocks.

Soil physical and chemical characteristics were analyzed as described in [70]. Simply, soil organic matter (SOM) was determined by the method of potassium dichromate heating oxidation-volumetric. Total nitrogen (TN) and total phosphorus (TP) were determined with a SAN++ system flow injection analyzer after digesting. Available nitrogen (AN;  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) was measured using SAN++ system flow injection analyzer after extracted with KCL. Available phosphorus (AP) was extracted with sodium bicarbonate and determined by Olsen method. Soil moisture (SM) was measured gravimetrically and pH data obtained from pH meter after dissolve soil into ddH<sub>2</sub>O. Climatic variables, including mean annual temperature

(MAT) and mean annual precipitation (MAP), for each sampling site were obtained using its coordinates from the WorldClim database ([www.worldclim.org](http://www.worldclim.org)).

### **Illumina sequencing of the 16S rRNA gene**

Total genomic DNA was extracted from soil samples according to MoBio Power Soil DNA isolation protocol (MO BIO laboratories, Inc, USA) using 0.25 g soil. Bacterial and archaeal 16S rRNA gene were amplified by the primer pairs 338F (ACTCCTACGGGAGGCAGCAG) / 806R (GGACTACHVGGGTWTCTAAT) [71] and Arch344F(ACGGGGYGCAGCAGGCGCGA) / Arch915R (GTGCTCCCCCGCCAATTCCT) [72] combined with adapter sequences and barcode sequences, respectively. The Purified PCR amplicons products were pooled in equimolar and paired-end sequenced on an Illumina MiSeq platform (Majorbio, Shanghai) according to the standard protocols. The acquired sequences were quality-filtered using QIIME (version 1.17). Chimeric sequences were identified and removed using the USEARCH in the UCHIME algorithm (Edgar et al., 2011). Operational taxonomic units (OTUs) with 3% dissimilarity cutoff were clustered using UPARSE pipeline (version 7.1) [73]. Those OTUs with less than two sequences were removed, and their representative sequences were classified within the SILVA database release 128 for bacteria and archaea.

### **Statistical analyses**

We respectively identified the abundant and ubiquitous microbes present in soil bacterial and archaeal communities on the Tibetan Plateau according to [32]. The Spearman's correlations between each two OTUs were estimated. The connection stands for a strong (Spearman's  $r > 0.6$ ) and significant (false discovery rate-corrected  $P < 0.01$ ) correlation were identified to construct networks in which each node represents one OTU and each edge represents a strong and significant correlation between two nodes. We generated sub-networks for each soil sample by subgraph function, while network-level and node-level topological features were calculated in the "igraph packages" in R [74]. Networks were visualized using the interactive Gephi platform [75]. Additionally, to test the potential roles of soil pH in bacterial and archaeal community assemblage, we clustered the abundant and ubiquitous bacterial and archaeal taxa into high-pH or low-pH cluster based on correlation coefficient.

A random forest analysis was applied to identify the major environmental factors contributing to the variation in network-level topological features. The analysis was performed using the randomForest function in the "randomForest" package in R [76]. We used a3 function to examine the significance values of the cross-validated  $R^2$  in the "A3" package in R. Moreover, the significance of each predictor on the response variables was assessed with 5000 response variable permutations using the rfPermute function of the R "rfPermute" package. A multiple regression model with variance decomposition analysis was applied by using the lm and calc.relimp function in the "relaimpo" package [77].

We used a kriging interpolation method to estimate the geographic patterns of the co-occurrence network-level topological features and the relative abundance of each ecological cluster for soil microbiota in alpine soils, respectively. The analysis was performed by the "automap" package in R [78], which

performs the interpolation process by automatically estimating a semivariogram and performing kriging. We used `autoKrige.cv` function to cross-validated our maps in the “`automap`” package in R [78], which automatically isolates the model structure dataset. We extracted the predicted values for each soil sample sites and tested their correlation with the observed values of the corresponding soil sites based on Pearson correlation analysis (`cor.test` function in “`stats`” package in R [79]). The Pearson correlation coefficient and *P* value were shown in the map.

All statistical analyses were conducted in the R environment (v3.6.1; <http://www.r-project.org/>), using “`vegan`” [80], “`igraph`” [74], “`stats`” [79], “`ggplot2`” [81], “`fdrtool`” [82], “`automap`” [78], “`randomForest`” [76], “`relaimpo`” [77] package, unless otherwise indicated.

## Declarations

### Additional files

**Table S1.** Description of node- and network-level topological features in the co-occurrence network for soil microbiota. **Table S2.** Multiple regression analysis to explain the variation caused by environmental factors in network-level topological features of the co-occurrence network for soil microbiota in the Tibetan alpine grasslands. **Table S3.** ANOVA of environmental factors correlated with dominant bacterial  $\beta$ -diversity in the Tibetan alpine grasslands. **Table S4.** ANOVA of environmental factors correlated with dominant archaeal  $\beta$ -diversity in the Tibetan alpine grasslands. **Table S5.** List of microbiota found in most common and ubiquitous bacterial and archaeal communities in alpine grasslands of the Tibetan Plateau (ZIP 146 kb).

**Fig S1.** Geographical location of the sampling sites in alpine grasslands of the Tibetan Plateau. **Fig S2.** The degree distribution of the co-occurrence network for soil microbiota in the Tibetan alpine grasslands. **Fig S3.** The degree distribution pattern of the co-occurrence network for soil bacteria and archaea and the dominant taxa assigned to high- and -low pH clusters in the Tibetan alpine grasslands. **Fig S4.** Correlation matrix between environmental factors and network-level topological features of the co-occurrence network for soil microbiota in the Tibetan alpine grasslands **Fig S5.** Comparison of environmental factors between the Tibetan alpine meadow and steppe ecosystems. **Fig S6.** Spatial distribution of soil pH and the density, degree centralization, eigenvector centralization, modularity, and positive edge number within or between kingdoms of the co-occurrence network for soil microbiota in the Tibetan alpine grasslands. **Fig S7.** The random forest mean predictor importance (percentage of increase in mean square error [MSE]) of environmental factors as drivers of network-level topological features of the co-occurrence network for soil microbiota in the Tibetan alpine grasslands. **Fig S8.** Dominant taxa (genera) of soil bacteria (blue) and archaea (red) assigned to the high- and low-pH clusters in the Tibetan alpine grasslands. **Fig S9.** Unique node-level topological features of the dominant bacterial and archaeal taxa assigned to high- and low-pH clusters in the Tibetan alpine grasslands. **Fig S10.** Correlation matrix between environmental and geographical factors in the Tibetan alpine grasslands. (PDF 1.4 Mb)

## **Ethics approval and consent to participate**

Not applicable.

## **Consent for publication**

Not applicable.

## **Availability of data and material**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## **Completing interests**

The authors declare that they have no competing interests.

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## **Authors' contributions**

XM and GD conceived the ideas and designed the experiments. BM and WQ collected the samples. BC, BM and CC carried out the experimental work. BC conducted High-throughput sequencing analysis. BC and SJ wrote the manuscript with contributions from all authors. All authors read and approved the manuscript before submission.

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## Tables

**Table 1** Partial Mantel test results for the correlation between network-level topological features and environmental variables in the Tibetan alpine grasslands

Variable <sup>a</sup>	Partial Mantel statistic r (P value) for <sup>b</sup> :	
	Network-level topological features	
pH	0.66	(0.001 <sup>***</sup> )
SM %	0.16	(0.001 <sup>***</sup> )
SOM %	0.21	(0.001 <sup>***</sup> )
TN (mg/g)	0.058	(0.02 <sup>*</sup> )
TP (mg/g)	0.24	(0.001 <sup>***</sup> )
AP (mg/kg)	0.073	(0.004 <sup>**</sup> )
NO <sub>3</sub> <sup>-</sup> -N (mg/kg)	0.084	(0.002 <sup>**</sup> )
NH <sub>4</sub> <sup>+</sup> -N (mg/kg)	0.091	(0.001 <sup>***</sup> )
MAT (°C)	0.059	(0.004 <sup>**</sup> )
MAP (mm)	0.32	(0.001 <sup>***</sup> )

<sup>a</sup>pH, soil pH; SOM, soil organic matter; AP, soil available phosphorus; TN, total nitrogen; TP, total phosphorus; SM, soil moisture content; NO<sub>3</sub><sup>-</sup>-N, nitrate-nitrogen; and NH<sub>4</sub><sup>+</sup>-N, ammonium-nitrogen; MAT, mean annual temperature; and MAP, mean annual precipitation

<sup>b</sup>Statistical significance was tested based on 999 permutations. Asterisks represent significance of correlation (\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ ).

**Table 2** Mantel and partial Mantel test results for the correlation between network-level topological features and environmental or geographical distance in the Tibetan alpine grasslands

Effect of	Mantel statistic r (P value) for <sup>a</sup> :	
	Controlling for	Network-level topological features
Environmental distance		0.31 (0.001 <sup>***</sup> )
Geographical distance		0.075 (0.001 <sup>***</sup> )
Environmental distance	Geographical distance	0.31 (0.001 <sup>***</sup> )
Geographical distance	Environmental distance	-0.075 (n.s.)

<sup>a</sup>Statistical significance was tested based on 999 permutations. Asterisks represent significance of correlation (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ; and n.s.,  $P > 0.05$ ).

**Table 3** Partial Mantel test results for the the correlation between dominant microbial community similarity and environmental variables in the Tibetan alpine grasslands

Variable	Partial Mantel statistic r (P value) for <sup>b</sup> :	
	Bacteria	Archaea
pH	0.70 (0.001 <sup>***</sup> )	0.64 (0.001 <sup>***</sup> )
SM %	0.28 (0.001 <sup>***</sup> )	0.16 (0.001 <sup>***</sup> )
SOM %	0.40(0.001 <sup>***</sup> )	0.21 (0.001 <sup>***</sup> )
TN (mg/g)	0.18(0.001 <sup>***</sup> )	0.073 (0.01 <sup>**</sup> )
TP (mg/g)	0.26 (0.001 <sup>***</sup> )	0.20 (0.001 <sup>***</sup> )
AP (mg/kg)	0.13 (0.001 <sup>***</sup> )	0.090 (0.001 <sup>***</sup> )
NO <sub>3</sub> <sup>-</sup> -N (mg/kg)	0.059 (0.005 <sup>**</sup> )	0.090 (0.002 <sup>**</sup> )
NH <sub>4</sub> <sup>+</sup> -N (mg/kg)	0.084 (0.001 <sup>***</sup> )	0.066 (0.007 <sup>**</sup> )
MAT(°C)	0.21 (0.001 <sup>***</sup> )	0.12 (0.001 <sup>***</sup> )
MAP(mm)	0.36 (0.001 <sup>***</sup> )	0.31 (0.001 <sup>***</sup> )

<sup>a</sup>pH, soil pH; SOM, soil organic matter; AP, soil available phosphorus; TN, total nitrogen; TP, total phosphorus; SM, soil moisture content; NO<sub>3</sub><sup>-</sup>-N, nitrate-nitrogen; and NH<sub>4</sub><sup>+</sup>-N, ammonium-nitrogen; MAT, mean annual temperature; and MAP, mean annual precipitation

<sup>b</sup>Statistical significance was tested based on 999 permutations. Asterisks represent significance of correlation (\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ ).

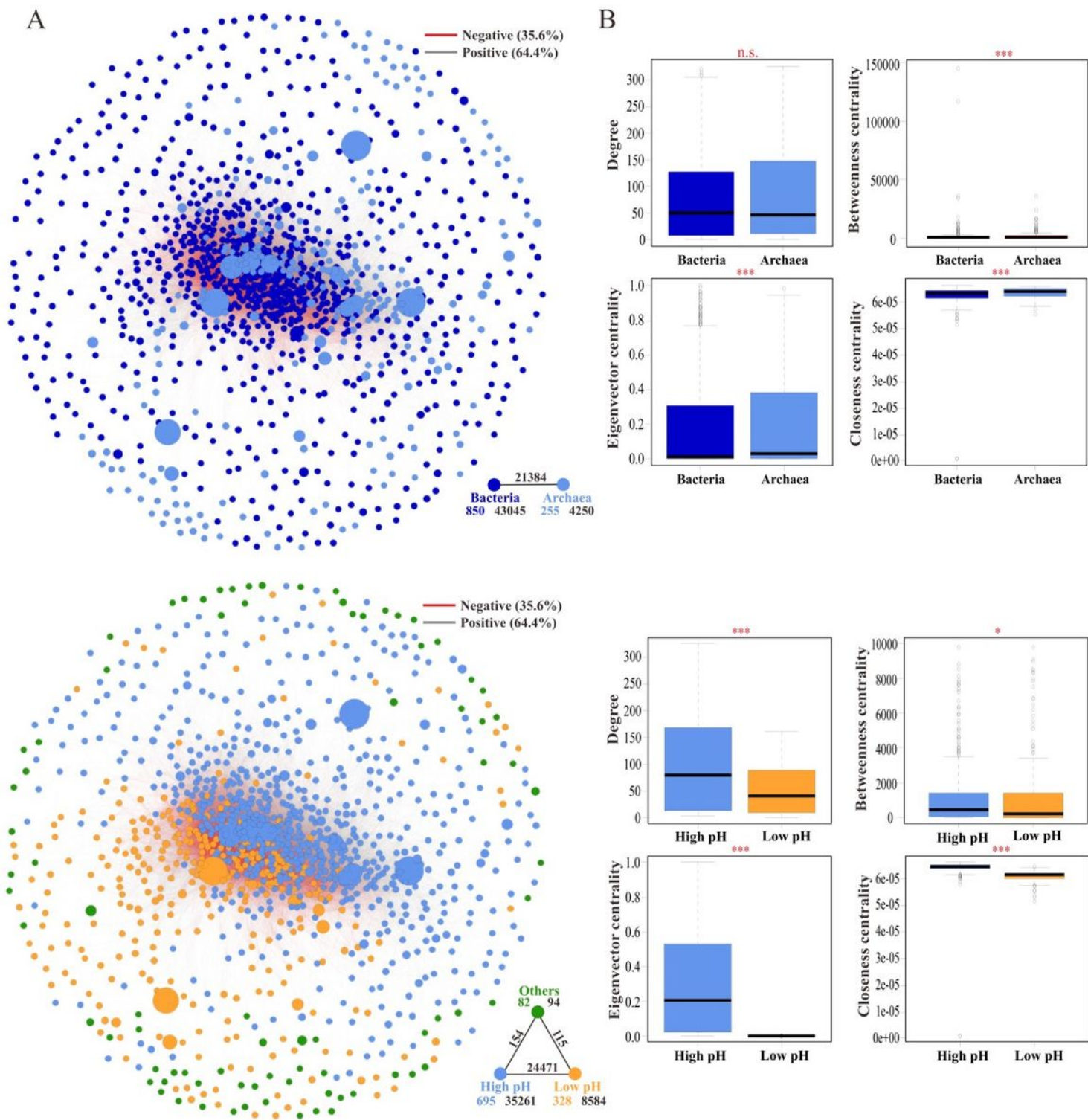
**Table 4** Mantel and partial Mantel test results for the correlation between dominant microbial community similarity and environmental or geographical distance in the Tibetan alpine grasslands

Effect of	Controlling for	Mantel statistic r (P value) for <sup>a</sup> :	
		Bacteria	Archaea
Environmental distance		0.41 (0.001 <sup>***</sup> )	0.34 (0.001 <sup>***</sup> )
Geographical distance		0.19 (0.001 <sup>***</sup> )	0.13 (0.001 <sup>***</sup> )
Environmental distance	Geographical distance	0.37 (0.001 <sup>***</sup> )	0.32 (0.001 <sup>***</sup> )
Geographical distance	Environmental distance	0.013 (n.s.)	-0.028 (n.s.)

<sup>a</sup>Statistical significance was tested based on 999 permutations. Asterisks represent significance of correlation (\* $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\* $P < 0.001$ ; and n.s.,  $P > 0.05$ ).

## Figures

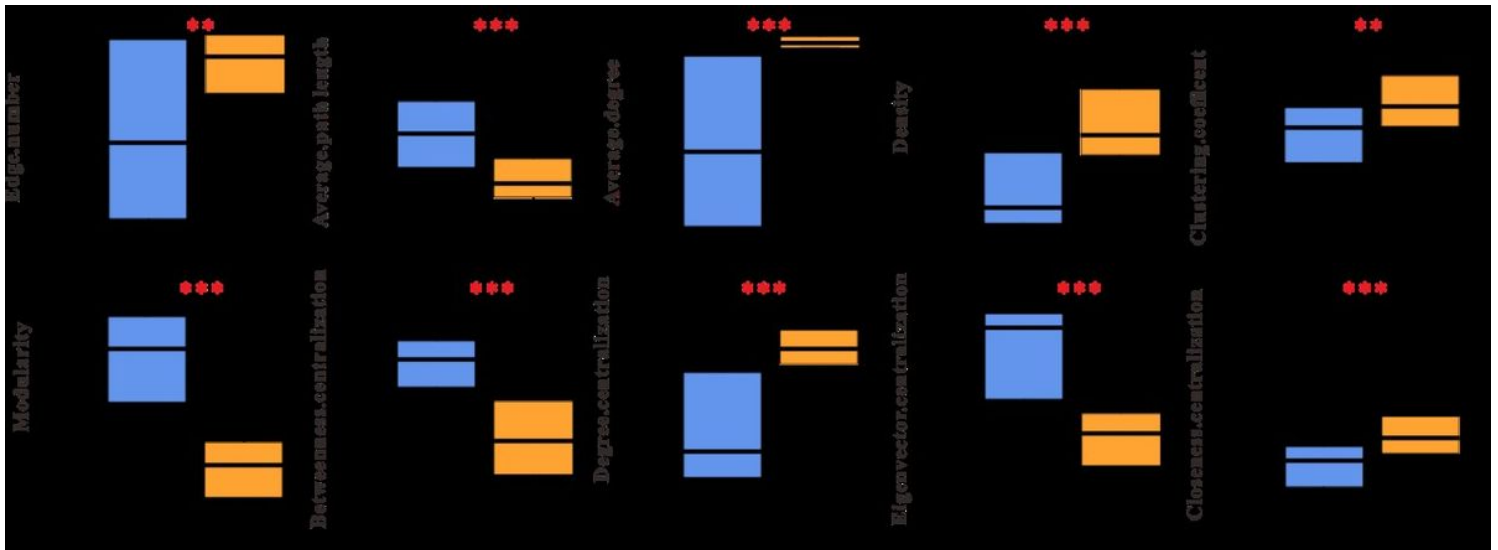




**Figure 1**

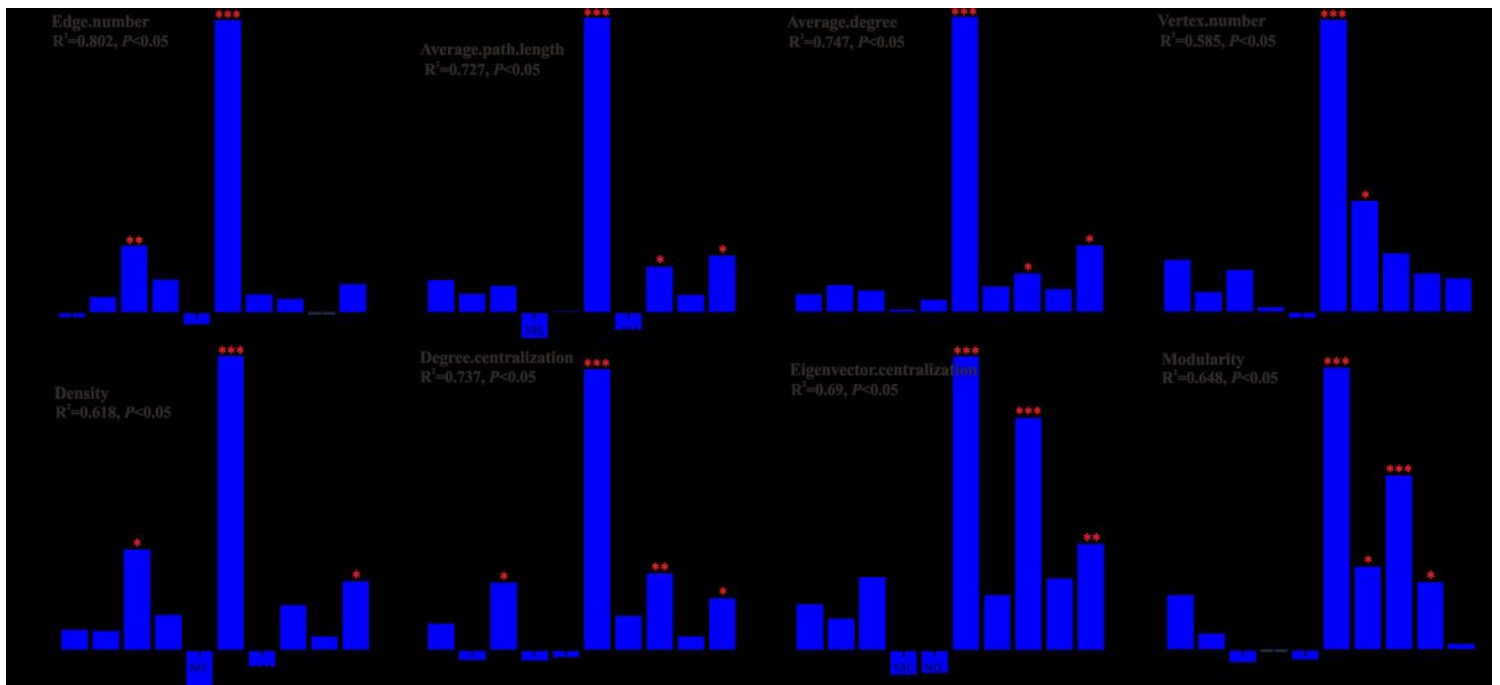
(A) Meta-community co-occurrence network with nodes colored according to dominant microbial taxa (top: bacteria and archaea) and ecological clusters (bottom: high pH, low pH, and others) of soil microbiota in the Tibetan alpine grasslands. The connection stands for a strong (Spearman's  $r > 0.6$ ) and significant (false discovery rate-corrected  $P < 0.01$ ) correlation. The size of each node is proportional to the relative abundance of the operational taxonomic units (OTUs) and the thickness of a connection

between two nodes (an edge) is proportional to the value of Spearman correlation coefficient. A red edge indicates a negative correlation and a gray edge indicates a positive correlation. A summary of node-edge statistics is provided to bottom right of the network. Colored numbers represent the number of nodes in corresponding categories, black numbers indicate the number of inner connections, and the numbers above edge connections represent the number of cross-group interactions. (B) Unique node-level topological features for dominant microbial taxa (top) and different ecological clusters (bottom). The degree, betweenness, closeness, and eigenvector centrality are shown. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  based on Wilcoxon rank sum tests.



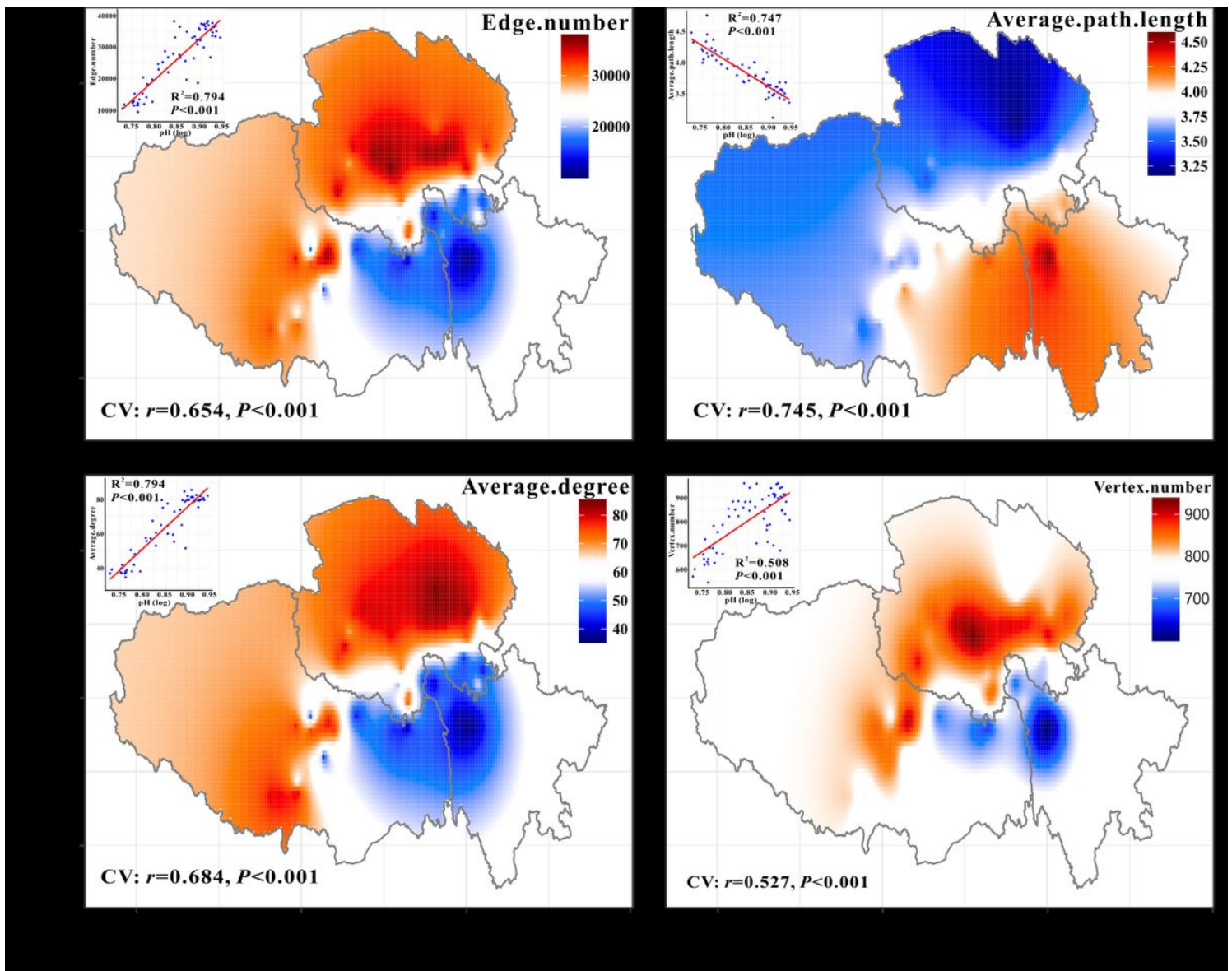
**Figure 2**

Network-level topological features of the meta-community co-occurrence network for soil microbiota in the Tibetan alpine meadow and steppe ecosystems. The edge number, average path length, average degree, density, clustering coefficient, modularity, betweenness centralization, degree centralization, eigenvector centralization, and closeness centralization are shown. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  based on Wilcoxon rank sum tests.



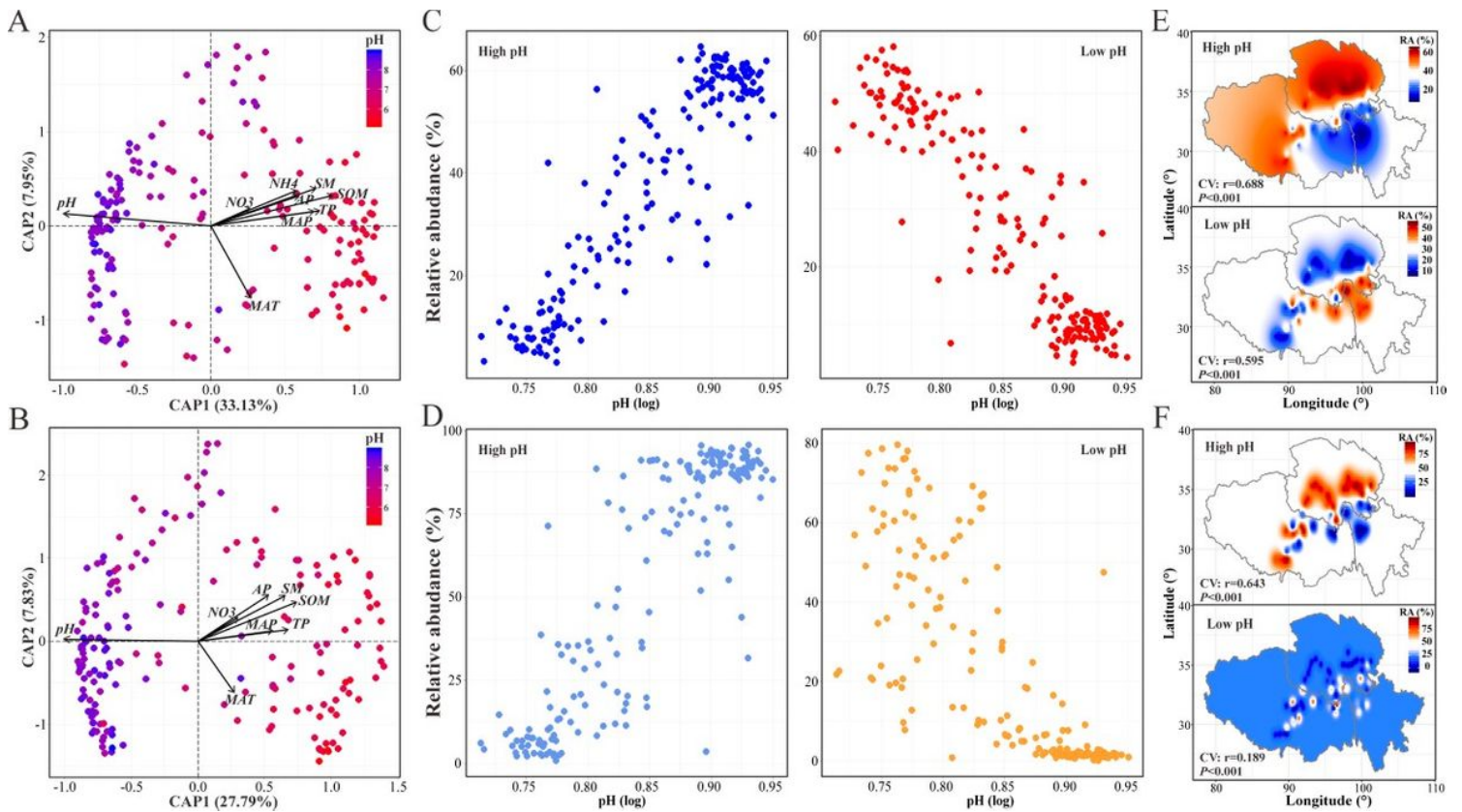
**Figure 3**

The random forest mean predictor importance (percentage increase in mean square error [MSE]) of environmental factors as drivers of the network-level topological features of the co-occurrence network for soil microbiota in the Tibetan alpine grasslands. The environmental factors studied include the available phosphorus (AP), mean annual precipitation (MAP), mean annual temperature (MAT), available nitrogen (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>), soil pH (pH), soil moisture (SM), soil organic matter (SOM), total nitrogen (TN), and total phosphorus (TP). The accuracy importance measure was computed for each tree. Percentage increases in the MSE of variables were used to estimate the importance of these predictors, and higher MSE% values indicate more important predictors. \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001.



**Figure 4**

The random forest mean predictor importance (percentage increase in mean square error [MSE]) of environmental factors as drivers of the network-level topological features of the co-occurrence network for soil microbiota in the Tibetan alpine grasslands. The environmental factors studied include the available phosphorus (AP), mean annual precipitation (MAP), mean annual temperature (MAT), available nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), soil pH (pH), soil moisture (SM), soil organic matter (SOM), total nitrogen (TN), and total phosphorus (TP). The accuracy importance measure was computed for each tree. Percentage increases in the MSE of variables were used to estimate the importance of these predictors, and higher MSE% values indicate more important predictors. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .



**Figure 5**

(A and B) Constrained analysis of principal coordinates (CAP) showing the environmental factors that shaped dominant bacterial (top) and archaeal (bottom) communities in the Tibetan alpine grasslands. The environmental factors shown include soil pH (pH), soil moisture (SM), soil organic matter (SOM), total phosphorus (TP), total nitrogen (TN), available phosphorus (AP), available nitrogen (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>), mean annual temperature (MAT), and mean annual precipitation (MAP). Sample points are colored according to soil pH (top right of each panel). (C and D) Relationships between soil pH and the relative abundance of dominant bacterial (top) and archaeal (bottom) taxa assigned to each ecological cluster. (E and F) The predicted distributions of dominant bacterial (top) and archaeal (bottom) taxa for each identified habitat preference. The cross-validation (“CV”) of the maps was calculated based on the Pearson correlation between the predicted and observed values at each sampling site.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfiles.docx](#)
- [TableS5.csv](#)