

Patterns in Plant-Microbiota Networks Along a Vegetation Diversity Gradient in Alpine Grasslands

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Abstract

Understanding how the network structure of plant and microbiota interactions differ along ecological gradients is of great interest. We studied network patterns at 60 sites across the Tibetan Plateau, representing a gradient in both precipitation and plant species richness. The number of fungal OTUs that were uniquely connected to each plant species in the plant-fungi network was most strongly positively related to plant species richness. By contrast, the number of unique bacterial OTUs linked to each plant species decreased with increasing plant species richness. The number of fungal OTUs specifically linked to each plant species was positively related to plant species richness, and to productivity. We suggest that in a more extreme high-stress environment that decreases plant species richness, plants and fungi have fewer excess resources to invest in specific relationships, showing up as lower associated microbiome richness, with bacteria may partially replacing this role in high stress/low productivity environments.

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Data accessibility statement

All the soil bacterial and fungal sequences data have been deposited, respectively, in the DDBJ Sequence Read Archive under accession number DRA001226 and the European Nucleotide Archive under accession number PRJEB16010.

Abstract

Understanding how the network structure of plant and microbiota interactions differ along ecological gradients is of great interest. We studied network patterns at 60 sites across the Tibetan Plateau, representing a gradient in both precipitation and plant species richness. The number of fungal OTUs that were uniquely connected to each plant species in the plant-fungi network was most strongly positively related to plant species richness. By contrast, the number of unique bacterial OTUs linked to each plant species decreased with increasing plant species richness. The number of fungal OTUs specifically linked to each plant species was positively related to plant species richness, and to productivity. We suggest that in a more extreme high-stress environment that decreases plant species richness, plants and fungi have fewer excess resources to invest in specific relationships, showing up as lower associated microbiome richness, with bacteria may partially replacing this role in high stress/low productivity environments.

Keywords: interkingdom and intrakingdom networks, connectivity, specificity, plant diversity gradient, Tibetan Plateau

Introduction

The impacts of biodiversity on ecosystem function are a classical and central topic in ecology (Bardgett & van der Putten 2014). Alpine plant and microbial communities are models for understanding pioneer communities, and the primary components of resilient ecosystems (Winkler *et al.* 2019). Relationships between plants and microbiota across environmental gradients could affect the ability of ecosystems to adapt to impacts such as climate change, by driving the maintenance biodiversity (Shen *et al.* 2021), community stability (Yang *et al.* 2022a), and ecosystem functioning (Wardle *et al.* 2004). Under natural conditions, the interactions between plant and microbial communities are complex, as they involve several factors that interact with each other and are subject to both biotic and abiotic influences, including temperature and water balance conditions (Cobian *et al.* 2019; Ware *et al.* 2021), plant species categories and distributions (Pölme *et al.* 2018; Yang *et al.* 2022b), and soil element nutrients (Zhu *et al.* 2022). However, most of these network studies on plant-microbiota interactions have focused on the rhizosphere microbiotas which vary among only one or a few distinct plant species (Toju *et al.* 2013; Toju *et al.* 2015; Polme *et al.* 2018; Yao *et al.* 2019), but it is still unclear how the traits of plant-microbial interactions vary along the plant diversity gradient.

With regard to the biogeographical patterns of soil microbiota, studies have been conducted on a range of scales, from global (Chu *et al.* 2020) to regional (Yang *et al.* 2021a) to the elevational zones of a single mountain (Yang *et al.* 2021b). These studies have noted patterns in relation to latitude (Fuhrman *et al.* 2008), climate (Yuan *et al.* 2021), soil characteristics (Chen *et al.* 2021), and land use (Xue *et al.* 2022) for example. However, few studies have considered the effects of plant richness and plant productivity on microbial cooccurrence network.

In this study, we apply cooccurrence network analysis to soil microbial communities and associated plants across an extensive area of the Tibetan Plateau samples. Cooccurrence networks are used to detect the possibility of specific interactions within communities (Barberan *et al.* 2012). This methodology empirically indicates the strength of interactions between species both positive and negative. The strength of these network interactions has been shown to correlate with the ability of the soil biota to carry out biogeochemical processes, and their resilience to environmental changes (Seaton *et al.* 2022).

Previous studies have shown that the plant diversity enhances soil fungal diversity in the natural environment of the Tibetan Plateau (Yang *et al.* 2017), as well as the soil fungal network stability in a diversity manipulation experiment (Shen *et al.* 2022). Here, we compared 60 sites along the precipitation gradient of the Tibetan Plateau, to qualitatively and quantitatively explore the proportions of multiple factors on different features of the cooccurrence network between plants and soil microbiotas. Using data from sampling carried out as part of a wide ranging study, we analysed amplicon data of the fungi (ITS2), bacteria (16S) and records of plant species present in each of 60 sites.

Our main question in this study was as follows: what are the cooccurrence relationships between plants and soil microbiota, and how does this vary along the environmental and plant richness gradients that are present?

We found a clear trend of decreasing richness of fungal OTUs associated with each plant species, along a gradient of decreasing total plant species richness. We suggest that this may reflect a decrease in reliance on specialised relationships by both plants and fungi due to reduced available energy in the more extreme plant species-poor environments. By contrast, the richness of specifically associated bacterial OTUs per plant species increased along this gradient, suggesting that bacteria may substitute as specialists in increasingly extreme environments associated with low plant species richness.

Methods

Sample collection and dataset information

Details on soil sampling, vegetation survey, measurement of soil properties, sequencing and bioinformatics were described in our previous study (Yang *et al.* 2017; Shi *et al.* 2019). Briefly, in the growing season of 2011, 180 soil samples were collected across the north-eastern and central Tibetan Plateau.

Within each of the sites, three of the ten small plots - each 1x1m in size - were randomly chosen on the diagonals of the 1-ha site at least 40 m apart (Fig S1). In each 1x1m plot, seven randomly located soil cores of the top 5 cm of topsoil (under any litter layer) were collected and homogenized as a composite soil sample. All vascular plant species and the number of individuals of each were recorded, at each of the 180 plots, and summarized at the site level (60 sites), and plant species richness was calculated.

All soil properties, including pH, soil moisture (SM), total carbon, total nitrogen, soil total phosphorus (STP), soil organic carbon (SOC), were measured at the individual plot level following (Jing *et al.* 2015), and then averaged at the site level. As three small plots were merged from each site, this gave 60 sites samples in total.

Soil DNA from each plot was extracted and amplified in the Illumina MiSeq platform PE250 (Illumina Inc., San Diego, CA, USA). The primers ITS3 (50-GCATCGATGAAGAACGCAGC)/ITS4 (50-TCCTCCGCTTATTGATATGC) were used to target the fungal internal transcribed spacer 2 (ITS2) region. The bioinformatic pipeline on ITS2 sequencing data was identical to the description of Teng *et al.* (Yang *et al.* 2017). The primer set of F515: 5'-GTGCCAGCMGCCGCGG, R907: 5'-CCGTCAATTCMTTTRAGTTT (Lane *et al.* 1985) were used to amplify the V4-V5 hyper-variable regions of bacterial 16S rRNA gene. The high-throughput sequencing process and analysis of soil fungi and bacteria has been described already by Teng *et al.* (Yang *et al.* 2017) and Shi *et al.* (Shi *et al.* 2019). In order to be compatible with plant and soil data, we merged the OTU table at the site level. Finally, 11,576,489 fungal sequences (min=123,753 sequences per site) and 1,031,092 bacterial sequences (min=9,037 sequences per site) were obtained in the 60 sites. In order to analyze the microbial data at the same sequencing depth, the OTU matrices were rarefied to 123,753 sequences per sample for fungi. But for bacteria, most sites were rarefied to 12850 sequences, only two sites far less than 12850 sequences were rarefied to 9037 reads, to keep as many reads as possible without compromising the bacterial alpha diversity. The richness of bacteria and fungi species were then calculated by “countif” function in Excel.

Network construction

To understand whether and how the co-occurrence networks including fungal and bacterial community varies across the Tibetan Plateau in relation to environmental and plant richness gradients, two different kinds of networks were constructed basing on the Spearman correlation matrix by “WGCNA” R package (Langfelder & Horvath 2012), viz., molecular ecological network (MEN) including fungi-only and bacteria-only network, and plant-microbiota interkingdom ecological networks (IDEN) including plant-fungi network and plant-bacteria networks.

Given the generally observed relationships between broad habitat zones and microbiota and floristic composition, we also divided the 60 samples according to the three main types of vegetation in our samples (desert steppe, alpine meadow, alpine steppe), and constructed plant-fungi and plant-bacteria networks for each vegetation type.

To avoid the bias of the correlation matrix causing by rare taxa, only OTUs with average relative abundances > 0.01% of each subgroup were retained. Since the number of plant species amongst these 60 sites is low, we kept all the plant species for the plant-microbiota network construction. The Spearman correlations between OTUs and plants were filtered by the thresholds $r > 0.6$ and false discovery rate adjusted $p < 0.05$ (Huang *et al.* 2019). The OTUs and plants presented at each site were retained and generated subnetworks for each soil sample from the combined interkingdom ecological networks by the “igraph” R package. Only the correlations between plants and fungi (or bacteria) in each site were kept by the “startswith” function in python, and were chosen as the adjacent matrix of the bipartite graph. The obtained adjacent matrix associated with the bipartite graph consisted of 1 or 0, showing the presence/absence of corresponding plant-microbiota associations (Feng *et al.* 2019). The plant-fungi and plant-bacteria network architecture of each group was visualized based on the “ForceAtlas2” layout algorithm (Jacomy *et al.* 2014) using the program Gephi (Bastian *et al.* 2009). We then examined the number of edges, plant and microbial species richness in the observed IDEN in 60 sites. The observed IDEN topological features (Table S1) was evaluated at both

network and group (plants or microbiota) levels using “bipartite” v.2.08 package of R v.3.1.1 (Dormann *et al.* 2009). Note that low Nestedness values indicate nestedness, while high Nestedness values (0 means cold, i.e. high nestedness, 100 means hot, i.e. chaos) indicate antinestedness. In a nested network, specialists (that is, species with narrow partner ranges) interact with subsets of the partners of generalists (that is, species with broad partner ranges) (Toju *et al.* 2015). To further determine the compartmentalization of the observed IDEN, modularity was calculated by module detection algorithm for example simulated annealing (Guimerà *et al.* 2005), and high value indicates modular structure. Modularity is a measure of the extent to which the network is structured as cohesive subgroups of nodes (modules), in which the density of interactions is higher within subgroups than among subgroups (Olesen *et al.* 2007).

We then conducted the microbial intrakingdom ecological networks analysis using the same thresholds for OTU and correlations mentioned above. To calculate the network-level topological features (Table S2), 60 subnetworks were generated by retaining the OTUs and associated edges for each site using the “subgraph” function in “igraph” R package. Network-level topological features with a high value (such as edge density, degree centralization and betweenness centralization) indicate closer connections within the network, whereas those with lower values (such as average path length and modularity) suggest a more aggregated network (Barberán *et al.* 2012; Ma *et al.* 2016). We then calculated the absolute value of negative/positive cohesion to explore the stability of microbial networks along gradient (Yuan *et al.* 2021).

Statistical analyses

To assess how different environmental factors shaped the network parameters of IDEN and MEN, we used cluster analysis to assess the collinearity or redundancy of environmental variables by the “varclus” function in the “Hmisc” R package before further analyses. Only one variable was selected if pairs of factors were highly correlated (Spearman’s $R^2 > 0.6$) as the representative variable. Using these criteria, MAP, MAT, carbon: nitrogen (C: N) ratio, SOC, STP, pH, SM, fungal richness, bacteria richness, plant richness and plant productivity were reserved in our analysis (Fig S1). Those environmental factors of 60 samples in desert steppe, alpine steppe and alpine meadow were showed by box plots, based on Kruskal-Wallis tests (Fig S2).

A random forest analysis was applied to identify the major environmental factors contributing to the variation in network topological features. The analysis was performed using the “randomForest” function in the “randomForest” package in R (Svetnik *et al.* 2003). Using the “a3” function to examine the significance values of the cross-validated R^2 in the “A3” package; the significance of each predictor on the response variables was assessed with 2000 response variable permutations using the “rfPermute” function in the “rfPermute” package in R.

The correlation coefficients between topological features and environmental factors were calculated. The importance of environmental factors for topological features was estimated with multiple regression on distance matrices (MRM) in “ecodist” packages. The Euclidean distance matrices for environmental factors and topological features standardized with “decostand” of “vegan” package were used in MRM models. We furthermore quantified the relationship between these factors and topological features by linear fitting.

To assess the variation trend in function of microbial nodes along the plant richness gradient, we divided sites into three groups of low (0-8 species per sites), medium (9-13 species per sites) and high (14-28 species per sites) plant richness. The function of microbiota linked to plants were predicted, and assigned to three groups through sites they belong to. Ecological guilds of fungal OTUs linked to plants were assigned using the “funguild.assign” function in the “FUNGuildR” package in R. Only sequence taxonomy identity above 97% and the guild confidence ranking assigned to ‘Highly probable’ and ‘Probable’ was accepted (Nuske *et al.* 2018). The PICRUSt 2 was used to predict the function (referring to KEGG pathway database) for each sequences of bacterial OTU linked to plants (Douglas *et al.* 2019).

Results

Patterns in plant-fungi relationships

A random forest analysis was performed to evaluate the major environmental factors to the topological features of plant-fungi network in the Tibetan Plateau. Plant species richness was the primary factor affecting the number of fungal OTUs links to each plant species and other topological features, such as the number of edges and modularity (Fig. 1a). These observations were also supported by heatmap based on spearman correlation (Fig. 1b) and the multiple regression on distance matrices analysis (Fig. 1c), confirming the leading effect of plant richness on the individual plant species to plant-fungi networks.

Further, there was an overall significant and positive linear relationship between plant species richness per site, and the number of fungal OTUs associated with each plant species in plant-fungi network (Fig 2a).

As a test of the possible explanation that there are only more fungal OTUs linked to each plant species because there is greater fungal richness in the whole soil community in the more plant species-rich samples, we calculated the ratio of fungal OTU richness to plant species richness and estimated its pattern along the plant species richness gradient. In fact, contrasting with the trend seen in Fig 2a for plant-linked OTUs only, the ratio of fungal OTUs in the total soil community to plant species decreased towards higher plant species richness levels (Fig 2b). Thus it appears that potential for association of fungi with plants enhances along the plant richness gradient unbiased by overall fungal richness in the soil, indicating that the tendency of fungi to form links with plants does actually increase with greater plant richness (Fig S3a).

The raising in plant richness increased the connection between plant and fungi at an alarming rate (Fig S3b), with the increasing number of plants (Fig S3c) and fungi (Fig S3d) in IDEN. The modularity and nestedness of the plant-fungi subnetwork in 60 sites also increased with increasing plant richness (Fig 2). While the proportions of functional guilds respectively belong to low, middle and high level of plant richness were similar in the plant-fungi network. (Fig S7a)

Patterns in plant-bacteria relationships

Plant richness only played a dominant role in the modularity in the plant-bacteria network, in contrast, SM was the key predictor for most of network parameters, the number of bacterial OTUs linked to each plant species (Fig S6).

The trend of the relationships in the plant-bacteria network was the opposite to that of the plant-fungi network: the trend in plant-bacteria links shows a greater number of bacterial OTUs linked to each plant species at lower levels of plant species richness (Fig 3a). Furthermore, the trend in the broader pool of total number of bacterial OTUs (in the whole soil community) plotted against plant species per site broadly paralleled this (Fig 3b).

In terms of the functional genes composition of bacterial OTUs in the network associated with the plants, carbon fixation, oxidative phosphorylation and methane metabolism were the dominant functional gene categories (Fig. S7b). While the proportion of functional genes in the plant-bacteria network that belong to low, middle, and high level of plant richness were similar.

Comparison by vegetation zone

The overall network pattern of each vegetation type along the gradient is summarized in Fig 3, illustrating the differences in numbers of OTUs linked to each plant species along the gradient from the semi-arid desert steppe, through alpine meadow to alpine steppe. For the plant-fungi networks, there was a mean of 36, 145 and 179 edges to fungi per plant species in desert steppe, alpine meadow and alpine steppe, including 0%, 15.17% and 25.14% of negative edges, respectively. There were more plant species in networks of both the alpine steppe (Fig 4c, 28 plant species) and alpine meadow (Fig 4b, 21 plant species), with each plant species associated with a greater diversity of fungi than in the semi-arid desert steppe (Fig 4a, 8 plant species).

There were fewer plant species in desert steppe (Fig 4d, 13 plants) and alpine steppe (Fig 4f, 6 plants) than meadow steppe (Fig 4e, 21 plants), while 306, 98 and 15 edges in desert steppe, alpine meadow and alpine steppe, including 0%, 19.39% and 33.3% of negative edges in the plant-microbial networks, respectively. The relatively few species of plants in desert steppe were thus associated with the widest range of bacteria.

Complexity of the soil microbial community

Complexity of the soil microbial community network (fungi-only network and bacteria-only network) showed distinct trends from those seen for the plant-microbiota networks, and were more strongly associated with soil factors than plant community parameters (Fig 5).

Random Forest analysis showed that the fungi-only network complexity was principally controlled by soil total phosphorus (STP) (Fig 5a&S8) while bacteria-only network complexity was dominated by soil moisture (SM) (Fig 5b&S9). In the fungal network, significant and positive linear correlations were found between STP and the number of nodes, number of edges, average degree, edge density, clustering coefficient, and degree centralization (Fig. 5c&S10). Significant and negative linear correlations were found between STP and average path length, modularity, betweenness centralization and eigenvector centralization (Fig. S10). The opposite trend was observed in bacterial networks, significant and negative linear correlations were found between SM and the number of nodes, number of edges, average degree, edge density, clustering coefficient, and degree centralization (Fig. 5d&S11). Significant and positive linear correlations were found between SM and average path length, modularity, betweenness centralization and eigenvector centralization (Fig. S11). These findings were supported by the results of the multiple regression on distance matrices analysis (Fig S12), indicating that increasing STP enhanced the network connections among fungi while increasing SM weakened the network connections among bacteria.

Discussion .

Plant richness enhances plant-fungi network

Our analysis showed a striking pattern in the numbers of fungal OTUs statistically associated with each plant species (Fig 2a), and revealed that in relatively plant species-poor plant samples, fewer fungal OTUs were specifically associated with each plant species. This cannot be explained simply by more fungal OTUs being present in the samples with high plant species richness: from the total ‘pool’ of fungal OTUs detected in the soil is very large in all samples, there are more fungal OTUs present - relative to each plant species - in the low plant species richness samples (Fig 2b).

It is unclear exactly what forms these specific plant-fungi interactions take, except that most are positive (81.39%) rather than negative (18.61%). Positive interactions could be mutualisms (Seidl *et al.* 2009), benefitting both sides, or specialised commensalisms benefitting only one side. Specialised pathogen-host associations may also be expected to show up as positive, unless they have a strongly depleting effect on host plant abundance. Negative interactions may occur across trophic levels involving plant defense mechanisms, or antagonistic relations with other fungal species that are specialised on the same plant host(Chen *et al.* 2018) .

Compared to the whole soil fungal community, the plant-associated fungi had a distinct profile of taxonomic identities and functional guilds (Fig S7a). The FUNGUILD classification reveals that around half of the OTUs in the fungi-plant networks are of unknown ecology, with putative saprophytes (22.67%), ectomycorrhizal fungi (4.04%) and plant pathogens (2.45%) dominating amongst those that could be assigned by FUNGUILD. In terms of the functional composition of the whole soil fungal communities, saprotrophs (30.5%) and plant pathogens (13.6%) were the dominant functional guilds. In alpine habitats, ectomycorrhizal (ECM) fungi are a significant functional guild, forming mutualisms with some small lignified shrubs and herbaceous perennials, and playing role in carbon and nutrient cycling.

The striking decrease in numbers of fungal OTUs specifically associated with each plant species, along the declining plant species richness gradient, suggests that interactions (both negative and positive) between plants and the rhizosphere biota are less intense in the more physiologically stressful environments associated with low plant richness. Plant species richness was strongly associated with a gradient in mean annual precipitation and plant productivity according to our previous study on this area(Yanget *et al.* 2017), so plant richness in fact could be seen as a general proxy for physiological stress on both plants and soil biota.

The trend in linked fungal OTUs per plant species thus suggests that under more stressful, low plant

diversity conditions, narrowly specific interkingdom interactions are rarer, and if anything are replaced by more generalised interactions that do not tend to produce detectable OTU-plant species links. This may be because the maintenance energy requirements for survival – on both plants and fungi – are greater, with less excess carbon and other resources available for engaging in specific interactions. For instance, an unpredictable flow of photosynthate from frequent drought conditions may also preclude niche specialisation by fungi on an unstable resource. The concept that physiologically extreme conditions preclude large numbers of specialised interactions has long been discussed, for example in explaining gradients in insect and plant diversity (Richards *et al.* 2015).

Different responses of fungi and bacteria to environmental stresses

The ratio of the whole pool of plants to bacterial OTUs in the community (Fig 3a) paralleled the trend in total fungal OTUs to plant species (Fig 3b). According to the results of PICRUST2, the functional guild assemblage of bacterial OTUs that were associated with plant species were dominated by carbon fixation and oxidative phosphorylation (Fig S7b), all of which are associated with energy metabolisms (Eida *et al.* 2018). Therefore, we suggest that soil bacteria communities may play a pivotal role in plant colonization in stressful environments by providing more available energy.

The findings of this study are of general interest as a novel perspective on how plant-microbial interactions may vary along environmental gradients, a broad and far reaching topic with many implications for ecosystem function. It would be very interesting to see if the same trends hold true along environmental or plant community gradients in other main ecosystems, such as forests, shrublands and agricultural landscapes. In addition, this study clearly showed the ecological differences between soil fungi and bacteria on the aspect of bipartite networks, although they had been known having the different pH niches (Rousk *et al.* 2010; Peay *et al.* 2016) and dependence on plants (Yang *et al.* 2019; Ni *et al.* 2021).

Conclusion

Along an broad gradient of environmental variables and plant species richness, number of fungal OTUs specifically linked to each plant species was positively related to plant species richness, while bacteria showed the opposite trend. We suggest that in a more extreme high-stress environment that decreases plant diversity, plants and fungi have fewer excess resources to invest in specific relationships, showing up as lower associated microbiome diversity, but that bacteria may partially replace this role of fungi in high stress/low productivity environments.

This appears to be the first instance in which an analysis of this type, finding plant-host links at the whole community level, has been carried out. It would be interesting to conduct similar studies in other ecosystems around the world.

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Figures and legends

Fig 1 Explanatory factors associated with features of the fungal-plant network in individual (**a** & **b**) and aggregate (**c**) (Only the top three factors are shown to be formally significant). (**a**) Random forest analysis showing influences on number of fungal OTUs linked to each plant species, number of edges and modularity of plant-fungi network. (**b**) The heatmap of spearman correlation coefficients between the network features and environmental factors. (**c**) The explained variation values were estimated with the multiple regression on distance matrices analysis. The results showed that plant species richness is the most important predictor for plant-fungi network topological features. MAP, mean annual precipitation; MAT, mean annual temperature; CNratio, carbon: nitrogen; SM, soil moisture; STP, soil total phosphorus; SOC, soil organic carbon. *P < 0.05, **P < 0.01, ***P < 0.001.

Fig 2 Relationship between plant species richness per site and plant-fungi network features (**a**, **c** & **d**) or the ratio of fungal richness to plant richness (**b**) in each site (vertical axis). (**b**) All OTUs detected in soil are included, not only those linked to plant species. (P = < 0.001)

Fig 3 Relationship between plant species richness per site and plant-bacteria network features (**a**, **c** & **d**) or the ratio of bacterial richness to plant richness (**b**) in each site (vertical axis). (**a**) there is no significant

trend between plant richness and number of bacterial OTUs linked to each plant species($P>0.05$). (b) All OTUs detected in soil are included, not only those linked to plant species. ($P=<0.001$)

Fig 4 Observed plant-microbiota network structure for each main vegetation type sampled in this study. In each network of (a) desert steppe (DS) (8 plants and 25 fungi), (b) alpine meadow (AM) (21 plants and 118 fungi), and (c) alpine steppe (AS) (28 plants and 138 fungi), (d) desert steppe (DS) (13 plants and 139 bacteria), (e) alpine meadow (AM) (21 plants and 84 bacteria), and (f) alpine steppe (AS) (6 plants and 15 bacteria), fungi (yellow) and bacteria (purple) are linked with their host plants (green). The size of circles represents the degree of microbiota or plants in each network. A blue edge indicates a negative correlation, and a red edge indicates a positive correlation.

Fig 5 Random Forest analysis and linear regression showing influences on the network-level topological features of fungi-only network(left) and bacterial-only network(right). Random forest shows that STP is the most important environmental factors for the fungi-only network (a), while SM is the most important for the bacteria-only network (b). MAP, mean annual precipitation; MAT, mean annual temperature; CNratio, carbon: nitrogen; SM, soil moisture; STP, soil total phosphorus; SOC, soil organic carbon. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Fig 1.

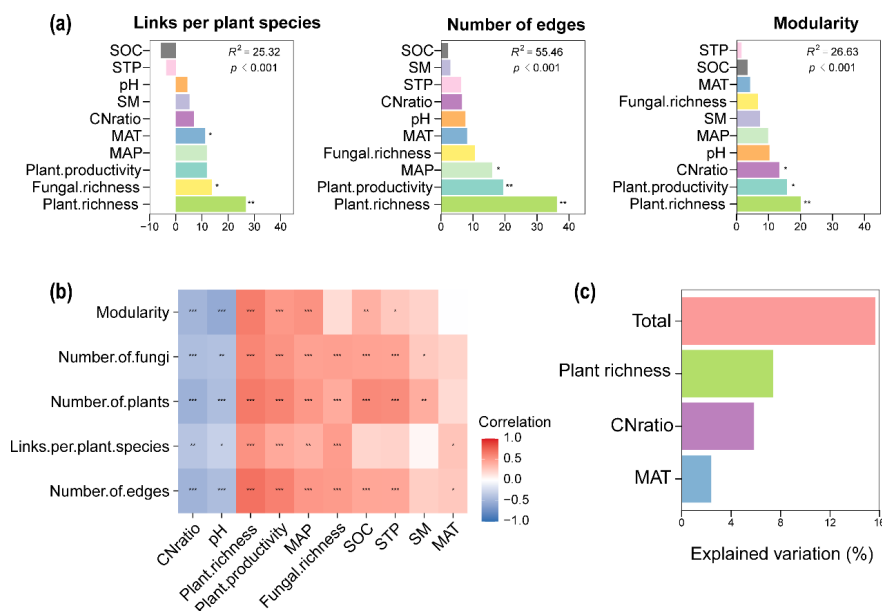


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Fig 2.

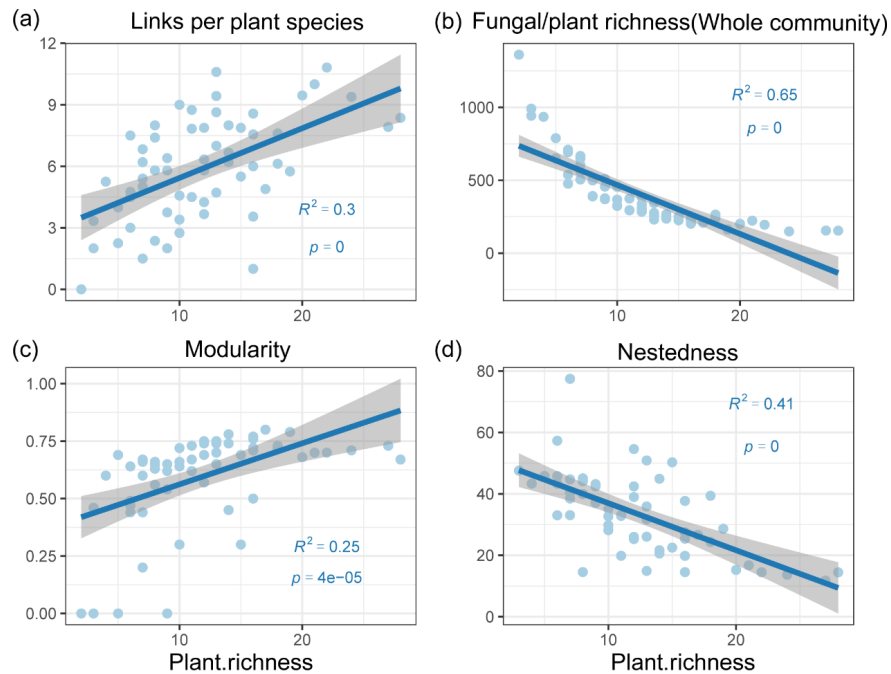


Fig 2 Relationship between plant species richness per site and plant-fungi network features (a, c& d) or the ratio of fungal richness to plant richness (b) in each site (vertical axis). (b)All OTUs detected in soil are included, not only those linked to plant species. ($P = < 0.001$)

Fig 3.

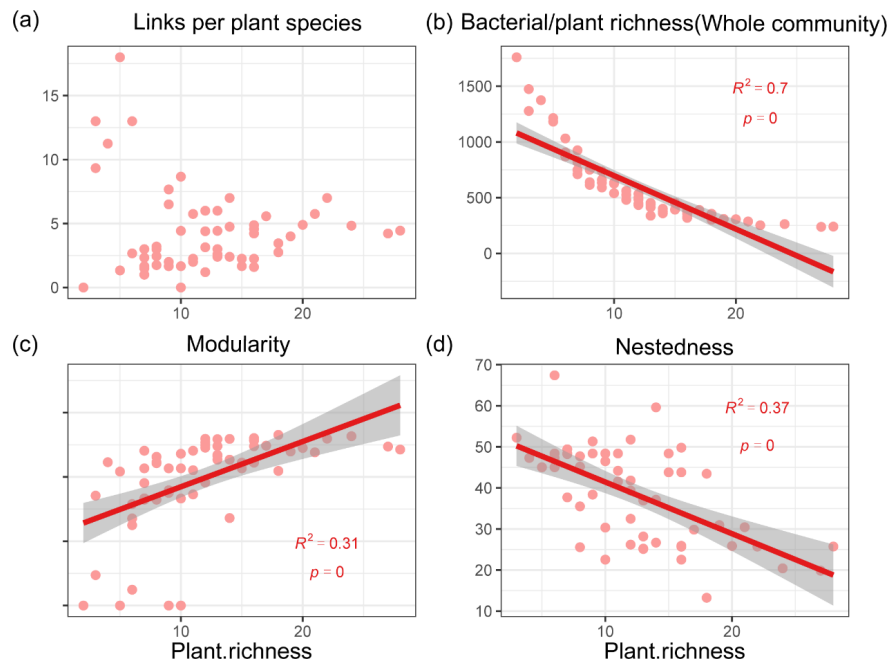


Fig 3 Relationship between plant species richness per site and plant-bacteria network features (a, c& d) or the ratio of bacterial richness to plant richness(b) in each site (vertical axis). (a) there is no significant

trend between plant richness and number of bacterial OTUs linked to each plant species ($P > 0.05$). (b) All OTUs detected in soil are included, not only those linked to plant species. ($P < 0.001$)

Fig 4.

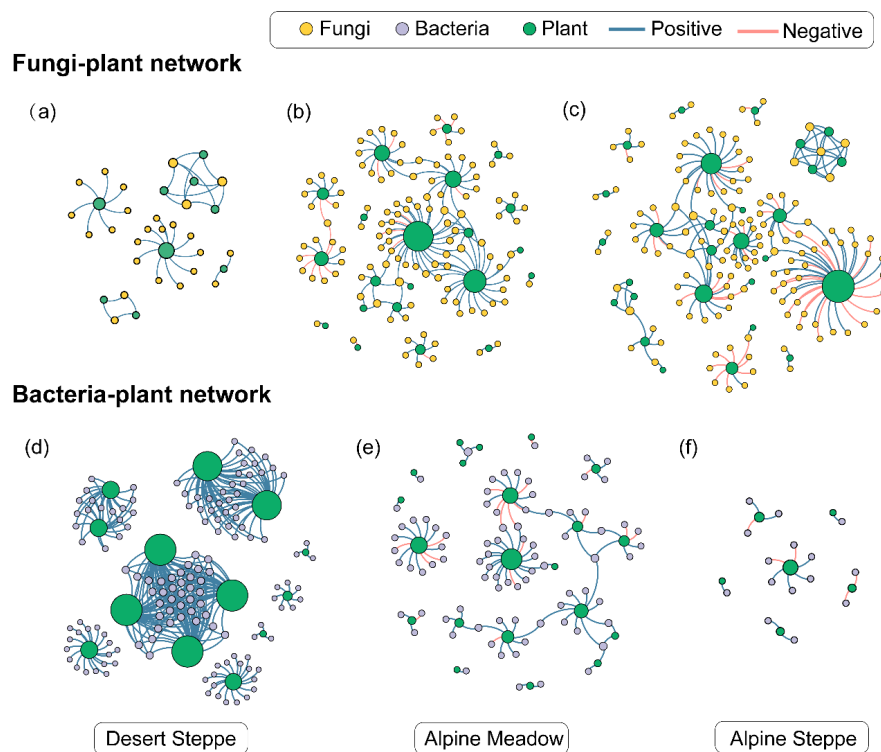


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Fig 5.

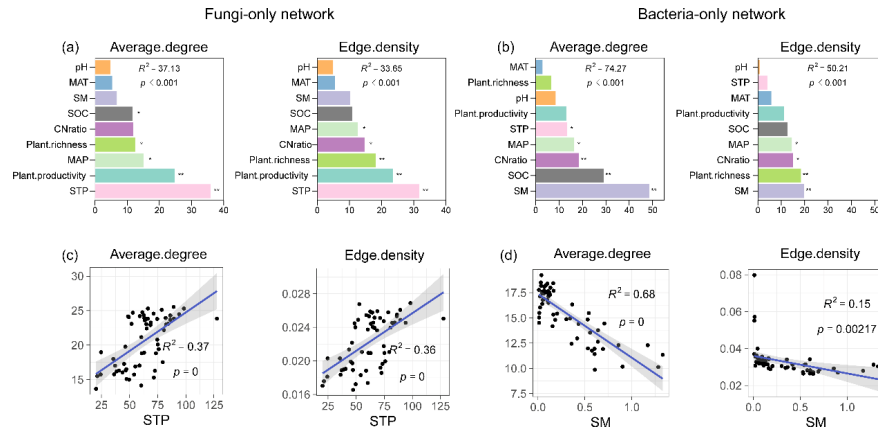


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