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Biogeographic patterns of microbial co-occurrence ecological networks in six American forests

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ABSTRACT

Rather than simple accumulation of individual populations, microorganisms in natural ecosystems form complex ecological networks that are critical to maintain ecosystem functions and services. Although various studies have examined the patterns of microbial community diversity and composition across spatial gradients, whether microbial co-occurrence relationships follow similar patterns remains an open question. In this study, we determined the biogeographic patterns of microbial co-occurrence networks of bacteria, fungi and nitrogen (N) fixer via analyses of high throughput amplicon sequencing data of 16S rRNA, ITS, and *nifH* genes from 126 forest soil samples across six forests in America. Microbial co-occurrence networks were constructed using a Random Matrix Theory based approach. Network parameters were calculated and correlated with biogeographic parameters. Gradient patterns along with biogeographic parameters were observed for network topologies. Significantly different network topologies were observed between microbial co-occurrence networks in tropical and temperate forest ecosystems. Among various biogeographic parameters potentially related with network topology indices, temperature seemed to be the strongest one. These results suggest that biogeographic variables like temperature not only mediate microbial community diversity and composition, but also the co-occurrence ecological networks among microbial species.

1. Introduction

Microbial communities play critical roles in various biogeochemical processes and determine ecosystem functioning (Fuhrman, 2009; Zavaleta et al., 2010; Miki et al., 2014; Wagg et al., 2014). Understanding the structure, composition, and distribution of soil microbial communities is therefore of critical importance to disentangle the mechanisms driving microbial community assembly. Various studies have suggested that microbial communities from different ecosystems are modulated by different biogeographic parameters (Martiny et al., 2006). For example, in soils, the diversity of free-living microbial taxa are strongly correlated with distance between sites (Cho and Tiedje, 2000), latitude (Staddon et al., 1998; Weiser et al., 2018), land use (McArthur et al., 1988; Buckley and Schmidt, 2003), pH (Fierer and Jackson, 2006; Griffiths et al., 2011), and multiple environmental variables (e.g. temperature, precipitation, pH and plant diversity) (Shay et al., 2015; Tu et al., 2016a; Zhou et al., 2016).

However, microbial species in natural ecosystems do not exist alone

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as individual populations. Rather, they interact with each other to form complex microbial communities and serve various ecosystem functions (Barberán et al., 2012; Montoya et al., 2006; Zhou et al., 2010). Therefore, our understanding of microbial communities should not only focus on the individual/species level characteristics such as species richness and abundance, but also more importantly on the interspecific characteristics of the complex microbial communities. The recently developed microbial co-occurrence ecological network analyses utilizing high throughput metagenomic data (Zhou et al., 2010, 2011; Barberán et al., 2012; Faust et al., 2012; Friedman and Alm, 2012; Xia et al., 2013) is an effective method that can be used to approximately characterize the interspecific relationships of complex microbial communities that cannot be observed directly (Fath et al., 2007), though these approaches may also suffer from a few limitations that both strength and weaknesses were found for different correlation methods (Weiss et al., 2016). Over the past years, much has been learned that microbial community diversity could be driven by multiple environmental/biogeographic factors. However, whether the complex interspecific characteristics of microbial communities are also shaped by any environmental/biogeographic factor is not yet clear.

Integrating network theory to biogeography is one of the most important and exciting challenges in macro-ecology (Cumming et al., 2010; Poisot et al., 2012). Over the past decades, ecological network studies (e.g. food webs, mutualistic and host-parasite networks) for macro-organisms along environmental gradients have been carried out, showing high variation in ecological networks in contrasting environments (Pellissier et al., 2018). For example, the specialization of plant-hummingbird interaction networks is positively correlated with warmer temperatures and greater historical temperature stability (Martín González et al., 2015). However, how microbial co-occurrence network characteristics (e.g. topologies) change across biogeographic gradients were rarely studied, until recently (Ma et al., 2016).

In this study, we aimed to investigate the potential biogeographic patterns followed by microbial co-occurrence ecological networks, thereby gaining further insights into the biogeography of complex microbial communities. Previous studies in the same experimental sites have shown clear gradient patterns of microbial diversity along temperature and/or latitude (Tu et al., 2016a; Zhou et al., 2016). In natural ecosystems, individuals in more complex communities are more likely to interact with other species. As a result, higher microbial diversity should be associated with more complex co-occurrence networks. We therefore hypothesize that: (i) tropical and temperate forest ecosystems have different microbial co-occurrence network topologies; and (ii) similar to what have been observed for microbial community diversity, a gradient pattern along temperature and/or latitude could also be observed for microbial co-occurrence networks.

2. Materials and methods

2.1. Sites and sampling

Six natural forest sites in America were surveyed in this study, including Barro Colorado Island, Panama (BCI); Luquillo Long Term Ecological Research (LTER), Puerto Rico (LUQ); Coweeta LTER, North Carolina (CWT); Niwot Ridge LTER, Colorado (NWT); Harvard Forest LTER, Massachusetts (HFR); and H.J. Andrews LTER, Oregon (AND). A total of 126 soil samples (0–10 cm, 21 samples per site) were collected from these six forest sites in the summer of 2012 for microbial community analysis. These selected sites provided variation in ecosystem types from boreal temperate to tropical forest, across a latitudinal gradient from 9 to 44°N. These sites were characterized by considerable soil and climatic variations with average annual temperature from 2.5 to 25.7 °C, plant species richness from 5 to 263 tree species, annual precipitation from 550 to 3460 mm, pH from 3.41 to 6.63, and soil moisture from 5.6% to 64.63%. More detailed description of climatic parameters and soil characteristics including soil type and texture, soil moisture, pH, total carbon, total nitrogen, and C/N ratio were also provided (Table 1). Details for experimental sites and sampling design could be found in supplementary data (Fig. S1). Soil DNA was extracted and purified as described previously (Zhou et al., 1996), subjected to PCR amplification for different regions (V4 region for 16S rRNA, ITS2 region for ITS, and *nifH*), and sequenced by Illumina MiSeq Platform. Negative controls using pure water was used during DNA extraction and PCR. The following primer pairs were used: 515F (5′-GTGCCAGCMG CCGCGGTAA-3′) and 806R (5′- GGACTACHVGGGTWTCTAAT-3′) for 16S rRNA gene, Pol115F (5′-TGCGAYCCSAARGCBGACTC-3′) and Pol457R (5′-ATSGCCATCATYTCRCCGGA3′) for *nifH*, and gITS7F (5′-GTGARTCATCGARTCTTTG-3′) and ITS4R (5′-TCCTCCGCTTATTG ATATGC-3′) for ITS. The *nifH* amplicon dataset representing soil diazotrophs was recruited for its strong correlation with biologically available nitrogen in the soil, indicating their potentially important roles in ecosystem functioning and stability (Tu et al., 2016a).

2.2. Data processing

Details for meta data collection and sequence processing could be found in our previous publications (Tu et al., 2016a; Zhou et al., 2016). Basically, raw data were first quality trimmed. Forward and reverse reads were then joined into longer sequences. OTUs were generated and identified using the UPARSE pipeline (Edgar, 2013). Potential chimeric sequences were checked against reference databases and removed by the UCHIME function included the UPARSE pipeline. An identity cutoff of 97% was used for 16S and ITS OTU clustering, while the identity cutoff used for OTU clustering of *nifH* sequences was 94%, which is the average nucleotide identity cutoff used for microbial species definition in post-genomic era (Konstantinidis and Tiedje, 2005). For 16S and ITS OTUs, taxonomic assignment was performed using the RDP pipeline. Taxonomic assignment of *nifH* OTU representative sequences was carried out using the Zehr *nifH* database (http://pmc.ucsc.edu/~wwwzehr/research/database/, 4 April 2014). A global identity cutoff of 90% was used for genus-level assignment.

2.3. Co-occurrence network construction, analysis and visualization

The microbial co-occurrence network was constructed based on 16S rRNA gene, ITS, and *nifH* gene amplicon sequencing data, representing prokaryotic-fungal-diazotrophic co-occurrence relationships in American forests. A random subsampling effort of 12,972, 7,724, and 8,000 sequences per sample was performed for 16S rRNA gene, ITS, and *nifH* gene amplicons, respectively. OTU relative abundance was calculated at each rarefied depth for each amplicon dataset. Microbial co-occurrence networks were constructed using the MENA pipeline, which implements Random Matrix Theory (RMT) to identify thresholds for constructing highly confident microbial ecological networks (Zhou et al., 2010; Deng et al., 2012). Briefly, Pearson correlation coefficients (r) was calculated between any two pairs of OTUs based on relative abundance values. OTUs presenting in 11 or more samples were selected for r calculation. The obtained similarity matrix was then transformed into an adjacency matrix. The RMT approach was then applied to determine the transition point of nearest-neighbor spacing distribution of eigenvalues from Gaussian (random) to Poisson (non-random) distribution, which are two universal extreme distributions. The transition point was then used as the threshold for co-occurrence network construction. Therefore, the main advantages of RMT approach lies in the power to remove noises from nonrandom by defining a threshold automatically based on the data structure itself rather than artificially chosen, and thus, no ambiguity occurs in constructing co-occurrence networks. The RMT approach is one of the most robust methodologies for confident co-occurrence network inference (Weiss et al., 2016), and has been widely applied to construct microbial co-occurrence patterns in various ecosystems (Zhou et al., 2010, 2011; Tu et al., 2015, 2016b; Deng et al., 2016; Ma et al., 2016).

Table 1
Summary of soil and climatic characteristics for the six sampling sites.

Sites	Latitude	Soil type and texture	Soil moisture[a] (%)	pH	Total carbon (mg/g)	Total nitrogen (mg/g)	C/N ratio	Mean temperature (°C)	Precipitation (mm)	Plant richness
AND	44.23	gravelly medial loam	36.88 ± 13.79	5.28 ± 0.64	19.36 ± 13.87	0.40 ± 0.18	42.9 ± 19.8	8.94	1587.4	17
HFR	42.54	fine sandy loam	34.35 ± 10.45	3.84 ± 0.29	19.21 ± 8.60	0.62 ± 0.29	31.2 ± 4.2	8.27	1128.7	25
NWT	40.04	cobbly silt loam	16.00 ± 8,74	4.83 ± 0.37	11.46 ± 7.03	0.33 ± 0.18	32.7 ± 6.2	2.5	481.6	5
CWT	35.05	gravelly loam	30.28 ± 6.05	4.72 ± 0.37	6.68 ± 4.07	0.25 ± 0.08	24.9 ± 5.3	12.62	1853.8	49
LUQ	18.32	clay	40.53 ± 4.42	5.06 ± 0.39	7.54 ± 3.05	0.47 ± 0.17	15.8 ± 2.1	23.62	3069.2	93
BCI	9.16	brown fine loam	31.43 ± 6.77	5.87 ± 0.42	3.99 ± 2.09	0.31 ± 0.12	12.3 ± 2.0	25.71	2383.0	263

[a] Mean and standard deviation of soil parameters (moisture, pH, total C, total N, and N/C) were calculated based on 21 samples collected in each site.

A global network was first constructed using data from all six forests sites. A minimum threshold of $r = 0.74$ was determined for co-occurrence network construction by the RMT approach. Although potential co-occurrence relationships were excluded for OTUs showing up in 10 or fewer samples, spurious and/or indirect correlations caused by insufficient samples were also less likely to occur. Inspection of *P* values for the calculated *r* of the constructed network showed they were all smaller than 0.002. Sub-networks representing the co-occurrence networks for each forest ecosystem were then extracted based on the criteria that each OTU should show up in 11 or more samples in each forest and *r* larger than 0.74.

To statistically evaluate whether the constructed networks are significantly different from random networks without ecological significance, a permutation based null model analysis was developed and applied (Zhou et al., 2010). Null models of random networks were constructed by fixing the node members and number of links, while randomizing the relationships between nodes. A total of 1000 random networks were constructed. One-sample *t*-test was used to evaluate whether network parameters (e.g. geodesic distance, clustering coefficient, and modularity) differed significantly between observed and random networks. Network topological parameters such as connectivity, geodesic distance, modularity, centrality of degree, centrality of betweenness, *Zi* and *Pi* values were all calculated using the *igraph* (Csardi and Nepusz, 2006) and *sna* (Butts, 2008) package in R. Additionally, we also used H-index of node connectivity as another index to measure the complexity of a network in this study. The Welch's *t*-test (unequal variances *t*-test) was used to statistically test whether network parameters were significantly different between tropical and temperate forest networks. Network modules were separated by the fast greedy modularity optimization process (Newman, 2006). Microbial co-occurrence networks were visualized by the Circos (Krzywinski et al., 2009) and Cytoscape (V3.2.1) (Shannon et al., 2003) software. To assess how microbial co-occurrence network parameters were shaped by different biogeographic parameters, Pearson correlation coefficient was calculated to estimate the relationship between network properties and environmental parameters.

2.4. Network terminologies

The following network parameters were used in this study and were therefore explained here:

Scale-free: scale-free is a network property that is commonly observed in social-networks. In a scale-free network, most nodes have few neighbors while only few nodes have large number of connected neighbors. As a result, the distribution of nodes connectivity follows a power law distribution.

Small-world: small-world is another property observed in social networks. In such a network, the average distance between two nodes is short, showing that the nodes in a network are always closely related with each other.

Average connectivity: Connectivity refers to the number of nodes directly connected by a node. It is the most commonly used concept for describing the topological property of a node in a network. Higher average connectivity usually means a more complex network.

Average geodesic distance: Geodesic distance is the shortest path between two nodes. A smaller average geodesic distance means all the nodes in the network are closer, therefore the network is more complex.

H index: Similar to the academic H-index definition (Hirsch, 2005), the H-index of node connectivity here is defined as the maximum value *h* such that there exists at least *h* nodes, each with *h* or more connections. Higher H-index suggests more nodes with high connectivity. The H-index of a network was calculated using an in-house developed PERL script according to the description in (Hirsch, 2005; Lu et al., 2016).

Modularity: modularity is the degree that a network can be divided into communities or modules. For ecological networks, microbial species in a module could be considered to have a similar ecological niche (Zhou et al., 2010). The value of modularity varies from 0 to 1. The higher modularity is, the more modules a network can be divided into and therefore the less complex a network is.

Degree centrality: Degree centrality is defined as the number of links incident upon a node. The value of degree centrality is close to 0 for a network where each node has the same connectivity. The value is closer to 1 when more differences were observed among the connectivity of all nodes. The higher value it is, the more complex a network is.

Betweenness centrality: Betweenness centrality quantifies the number of times a node acts as a bridge along the shortest path between two other nodes. The value of betweenness centrality is close to 0 for a network where each node has the same betweenness. The value is closer to 1 when more differences were observed among the betweenness values of all nodes. The higher value it is, the more complex a network is.

ZP-plot: We used ZP-plot to distinct the roles that each node play in the network by analyzing two parameters including within-module connectivity (Z_i) and among module connectivity (P_i). The roles of nodes can be classified into four different categories, including peripherals ($Z_i < 2.5$, $P_i < 0.62$), connectors ($Z_i < 2.5$, $P_i \geq 0.62$), module hubs ($Z_i \geq 2.5$, $P_i < 0.62$) and network hubs ($Z_i \geq 2.5$, $P_i \leq 0.62$). The threshold for Z_i and P_i can be referred in a previous literature (Guimerà and Nunes Amaral, 2005; Olesen et al., 2007; Oldham et al., 2008; Zhou et al., 2010).

Data availability: The raw sequence data for 16S rRNA gene, ITS, and *nifH* gene amplicons have been deposited under NCBI accession number PRJNA308872.

3. Results

3.1. An overview of the constructed microbial co-occurrence networks

The constructed consensus network was consisted of 1,251 OTUs,

including 1,065 16S OTUs, 127 *nifH* OTUs and 59 ITS OTUs. A total of 4,922 co-occurrence relationships were inferred for the consensus microbial network (Fig. 1A). Analyses of basic network properties suggested that the constructed network followed basic social network behaviors such as scale-free, small-world, and modular (Supplementary results). Null model analysis suggested that the constructed network properties were significantly different from random networks. These suggested that the constructed microbial co-occurrence networks were not random and could be considered being of biological significance as other biological networks. Analyses of subnetworks for diazotrophic and fungal communities suggested different co-occurrence patterns established by different microbial groups (Fig. 1B and C). Details describing the constructed co-occurrence networks can be found in the supplementary results.

3.2. Tropical and temperate forests exhibited distinct co-occurrence network topologies

To examine if co-occurrence network topologies were different in tropical and temperate forests, analyses were carried out at different angles, including nodes, links, and co-occurrence patterns. Three types of nodes were analyzed here for their potentially important roles in the co-occurrence network topologies, including nodes with high H-index and betweenness centrality, and keystone nodes. Distinct taxonomic profiles were observed between tropical and temperate forest microbial networks for the nodes with high H-index (Fig. 2A). Nodes with high H-index in tropical microbial networks were mainly OTUs belonging to *Acidobacteria*, *Proteobacteria*, and *Verrucomicrobia*, while the nodes with high H-index in temperate forest microbial networks were composed of OTUs from many different taxonomic groups. Among them, microbial OTUs belonging to *Sphingobacteriales*, *Actinomycetales*, *Rhodospirillales*, and *Caulobacterales* contributed as the major and/or unique nodes with high H-index in temperate microbial forest networks. Similarly, the taxonomic profiles for nodes with high betweenness centrality were also markedly different between tropical and temperate forest microbial networks (Fig. 2B). The nodes with high betweenness in tropical microbial networks were taxonomically more diverse than in temperate forest microbial networks. Although OTUs belonging to *Proteobacteria*, *Acidobacteria*, and *Verrucobacteria* dominated the nodes with high betweenness centrality, the relative numbers of *Proteobacteria* and *Acidobacteria* OTUs were quite different between tropical and temperate forest microbial networks. Specifically, more *Proteobacteria* nodes were found with high betweenness centrality in tropical microbial networks, while more *Acidobacteria* nodes with high betweenness centrality were found in temperate forest microbial communities. Interestingly, a relatively high number of *nifH* OTUs were found among the nodes with high betweenness centrality, especially in tropical ecosystems (Fig. 2C), indicating that soil diazotrophs may play important roles in connecting different microorganisms and transferring energy and resources.

Keystone nodes (module hubs, network hubs, and connectors) were identified by analyzing the topological roles that each node played in different networks (Fig. 3, Fig. S2). No network hubs ($Z_i \geq 2.5$, $P_i \geq 0.62$) were found in the networks for all six sites. We therefore mainly looked into the module hubs and connectors of different networks. A total of eight module hubs were found in both tropical networks (Fig. 3, Fig. S2), including four *Proteobacteria* OTUs (16S_18, 16S_637_*Syntrophobacterales*, 16S_1416_*Burkhoderiales,* and 16S_34706_*Rhizobiales*), two *Acidobacteria* OTUs (16S_136_*Acidobacteria* Gp5 and 16S_35039_*Acidobacteria* Gp6), one *Verrucomicrobia* OTU (16S_2_*Spartobacteria*) and one unclassified OTU (16S_15). One *Proteobacteria* OTU (16S_21280_*Rhodoplanes*) played a role of network connector in both tropical networks (Fig. 3, Fig. S2). For temperate forest networks (Fig. 3, Fig. S2), a total of nine module hubs were present in three or more networks, including four *Acidobacteria* OTUs (16S_10_Gp1, 16S_123_Gp1, 16S_21546_Gp6, and 16S_31892_Gp6), three *Proteobacteria* OTUs (16S_40_*Acetobacteraceae*, 16S_32512_*Gammaproteobacteria*,

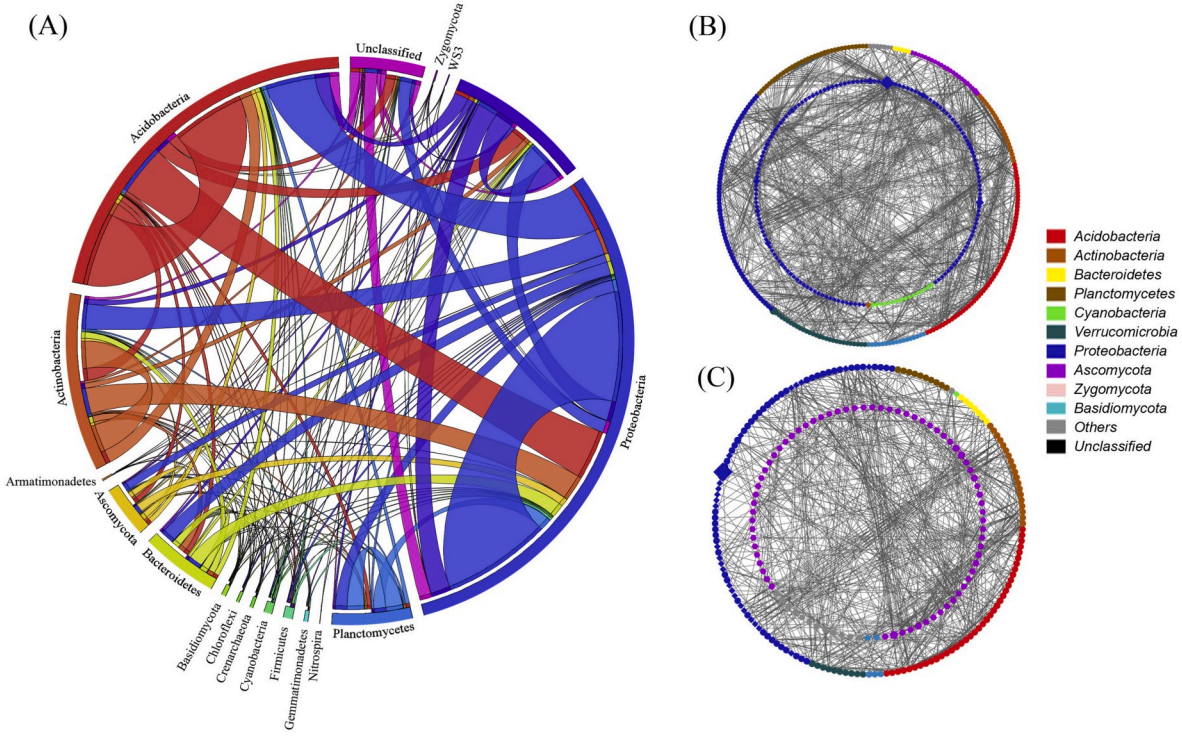

Fig. 1. The consensus network representing microbial co-occurrence networks across American forests. (A) An overall Circos illustration of the whole consensus network; (B) The sub-network centered by soil diazotrophs representing diazotrophic-microbial co-occurrence patterns; (C) The sub-network centered by soil fungi representing fungal-microbial co-occurrence patterns. The width of links within/between microbial phyla represents the number of connections between OTUs belonging to the connected phyla. Diamond and hexagon nodes in (B) and (C) are *nifH* and ITS OTUs, respectively.

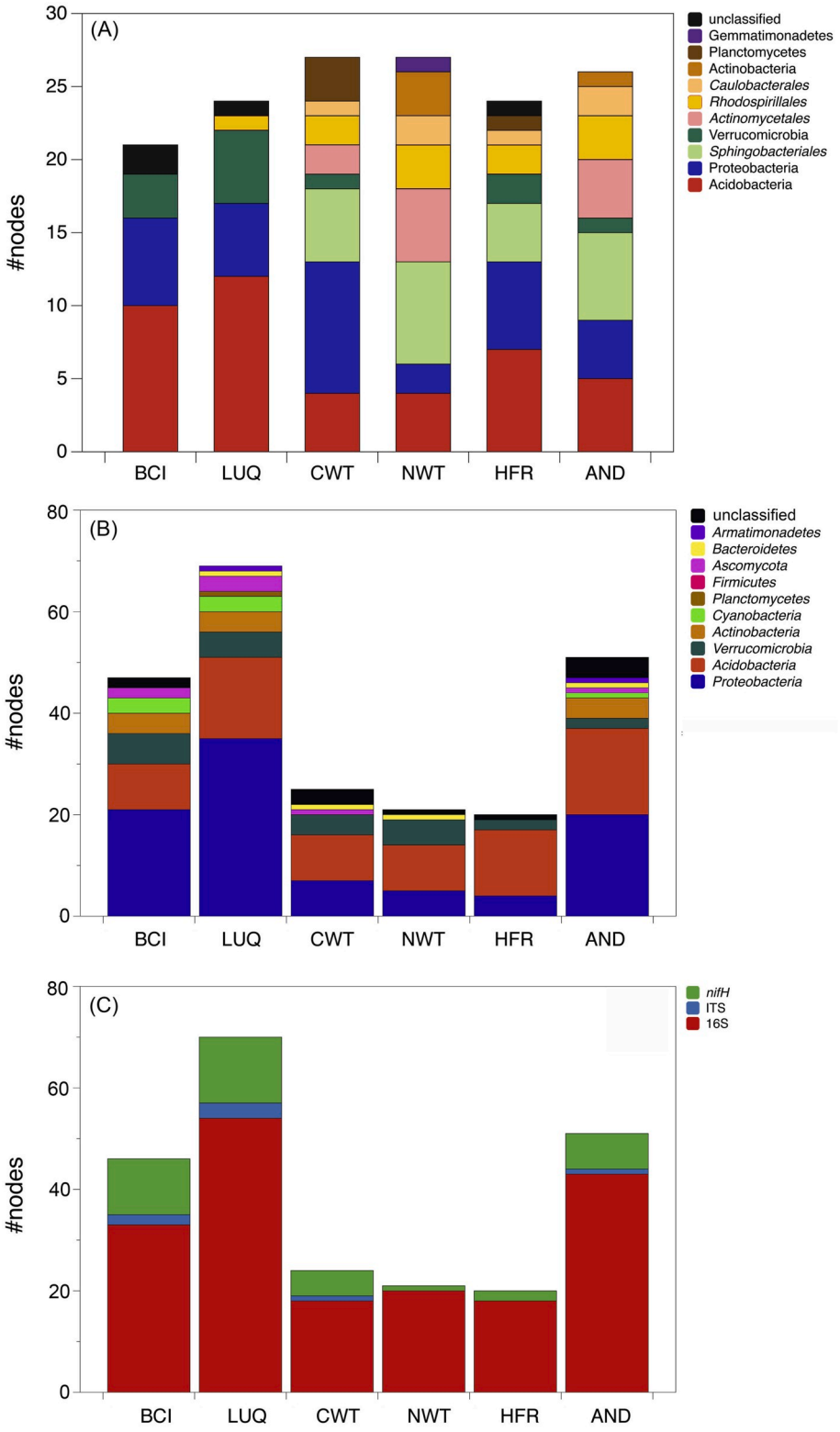

Fig. 2. Taxonomic composition of nodes with high H-index connectivity (A) and high centrality of betweenness (B), and gene composition of nodes with high centrality of betweenness (C). The nodes with high H-index in temperate forest microbial networks were more diversely composited with OTUs from many different taxonomic groups than those in tropical networks. The nodes with high centrality of betweenness in tropical microbial networks were taxonomically more diverse than that in temperate forest microbial networks. A relatively high number of *nifH* OTUs were found among the nodes with high centrality of betweenness, especially in tropical ecosystems.

and *nifH*_1_*Alphaproteobacteria*), one *Planctomycetes* OTU (16S_61_*Planctomycetaceae*), and one *Verrucomicrobia* OTU (16S_427_subdivision3). No common network connectors were found for temperate forest networks. The *nifH*_1 OTU belonging to *Alphaproteobacteria* was found as a module hub in five networks, showing the importance of this OTU in American forests. Module hubs rarely overlapped between tropical networks and temperate forest networks, showing that microbial species responsible for these topological roles are different between tropical and temperate forests.

Microbial co-occurrence patterns, including microbial-microbial (here microbial includes bacterial, fungal, and diazotrophic), fungal-microbial, and diazotrophic-microbial links, were also extracted and analyzed. The top ten most frequent co-occurrence patterns were analyzed (Fig. 4). No significant difference was found for the majority of

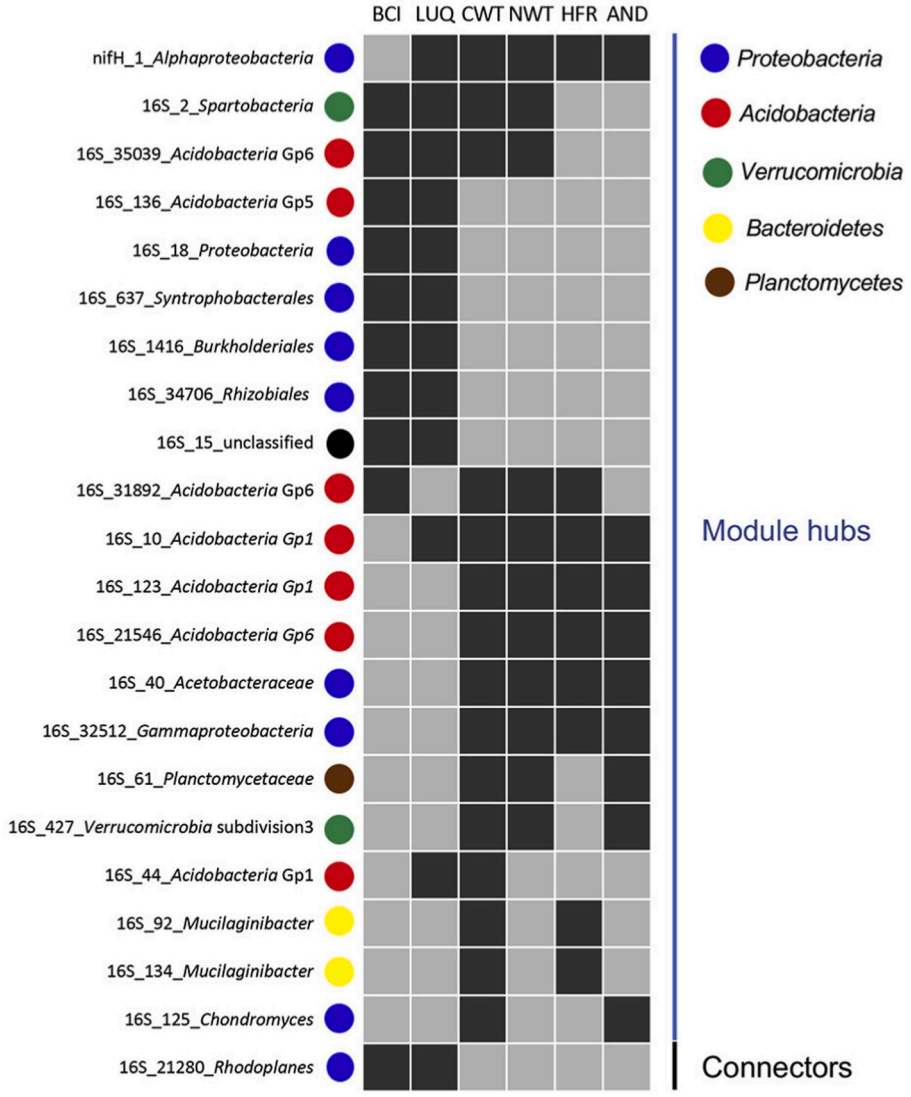

Fig. 3. Keystone nodes (module hubs, network hubs and connectors) showing up in two or more co-occurrence networks. No network hubs were found in this study. Black indicates presence, while gray indicates absense. Different colored circles represent different taxonomic groups.

bacterial-bacterial co-occurrence frequencies, except Proteobacteria-Proteobacteria, Bacteroidetes-Proteobacteria, Verrucomicrobia-Verrucomicrobia, and Acidobacteria-Actinobacteria (Fig. 4A). Of these, Proteobacteria-Proteobacteria and Verrucomicrobia-Verrucomicrobia patterns occurred more frequently in tropical microbial networks, while Bacteroidetes-Proteobacteria and Acidobacteria-Actinobacteria patterns were more frequently detected in temperate forest microbial networks. For fungal-microbial and diazotrophic-microbial patterns, significant differences could be observed between tropical and temperate forests (Fig. 4BC). Most fungal-microbial and diazotrophic-microbial patterns occurred more frequently in tropical microbial networks, except Acidobacteria-Basidiomycetes patterns, which were uniquely detected in temperate forest microbial networks. This suggests that fungi and soil diazotrophs could be more important in microbial co-occurrence networks in tropical forests than that in temperate forests.

3.3. Microbial co-occurrence networks in tropical forests were less complex than that in temperate forests

We then investigated the complexity of microbial co-occurrence networks in tropical and temperate forests (Fig. 5). The tropical and temperate forests did not differ significantly in network topological parameters such as average geodesic distance, network modularity and centrality of degree (Fig. 5E–G). However, tropical and temperate forests differed significantly or marginally significantly in the number of nodes, average connectivity, H-index of node connectivity, the number of links (Fig. 5A–D), and the centrality of betweenness (Fig. 5H). Specifically, microbial co-occurrence networks from temperate forests were found with 1264 ± 78 normalized number of links and 394 ± 9 normalized number of nodes, while the normalized number was 1,005 ± 36 links and 421 ± 5 nodes for tropical networks (Fig. 5AD). Consequently, temperate forest networks were found with significantly higher average connectivity than tropical networks (6.4 ± 0.27 vs. 4.77 ± 0.11) (Fig. 5B). The H-index of node connectivity was also significantly higher in temperate forest networks (H-index = 23.5 ± 1.0) than that in tropical networks (H-index = 18.5 ± 0.5) (Fig. 5C). Finally, the centrality of betweenness was also significantly higher in temperate forest networks (0.21 ± 0.03) than in tropical networks (0.11 ± 0.02) (Fig. 5H). Such results suggested lower complexity of microbial co-occurrence networks in tropical forests than that in temperate forests.

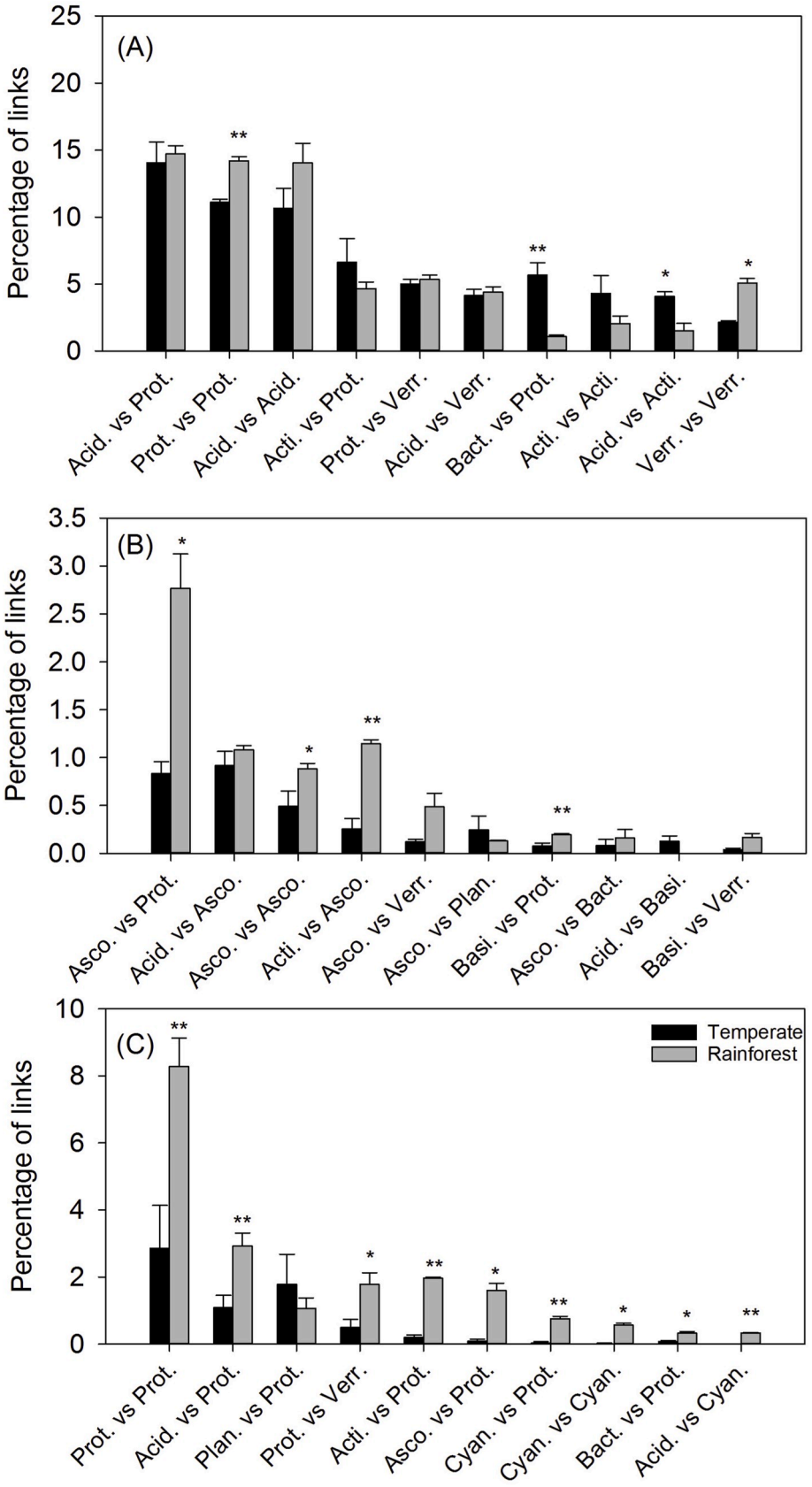

Fig. 4. Relative frequencies of the top ten most frequent co-occurrence patterns in tropical and temperate networks. Microbial-microbial (A), fungal-microbial (B), and diazotrophic-microbial (C) co-occurrence patterns were analyzed. Welch's *t*-test (unequal variances *t*-test) was used here. * indicates p value < 0.1, and ** indicates p value < 0.05.

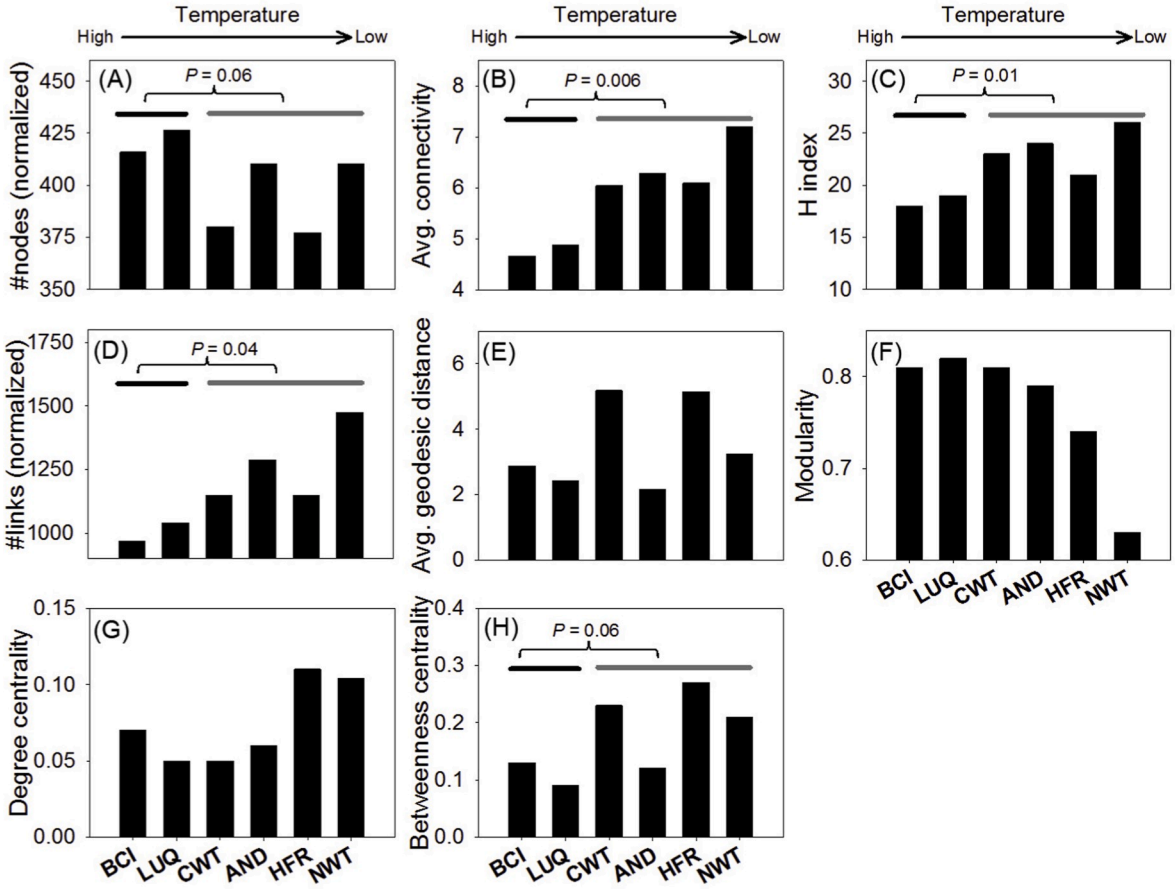

Fig. 5. Network parameters for microbial co-occurrence networks inferred in different forests. Major parameters including number of nodes (A), average connectivity (B), H-index (C), number of links (D), average geodesic distance (E), modularity (F), centrality of degree (G), and centrality of betweenness (H) were analyzed and plotted. Welch's *t*-test was carried out between tropical (BCI and LUQ) and temperate forest (CWT, NWT, HFR, and AND) networks.

3.4. Linkages between co-occurrence networks and biogeographic parameters

Finally, we assessed whether microbial co-occurrence networks vary with the same biogeographic variables that shapes macro- and microbial communities. We first noticed a clear trend of decreasing network complexity with increasing temperature (Fig. 5A). Such trend diminished when viewed by latitude. Interestingly, with the exception of average geodesic distance and centralization of degree, most network parameters we analyzed were significantly correlated with several biogeographic properties (Fig. 6). Specifically, average connectivity and H-index of node connectivity increased significantly ($P \leq 0.05$) with latitude (R = 0.88 and 0.83, respectively), decreased significantly ($P \leq 0.05$) with temperature (R = −0.99 and −0.91, respectively), soil NH_4^+ (R = −0.88 and −0.84, respectively), plant richness (R = −0.83 and −0.79, respectively, P < 0.1 for H index), plant diversity (R = −0.96 and −0.89, respectively), precipitation (R = −0.88 and −0.74, respectively, P < 0.1 for H index), and marginally ($P \leq 0.1$) with soil NO_3^- (R = −0.81 and −0.74, respectively). The network modularity, however, increased significantly ($P \leq 0.05$) with temperature (R = 0.86), precipitation (R =

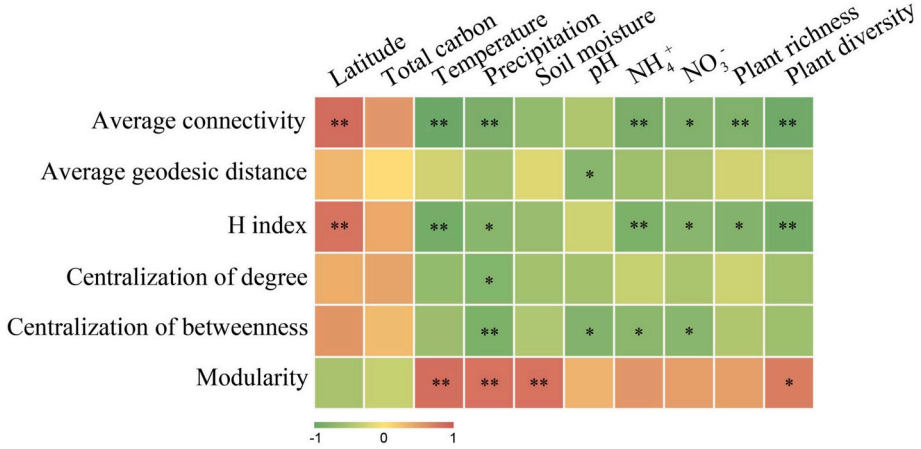

Fig. 6. Linkage between microbial co-occurrence network properties and biogeographic parameters. Different network parameters were correlated with different biogeographic properties, among which parameters related with temperature seemed to the strongest. Red indicates positive correlations, and green indicates negative correlations. * indicates *p* value < 0.1, and ** indicates *p* value < 0.05. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

0.83), soil moisture (R = 0.84), and marginally with plant diversity (R = 0.75). These results suggested that the complexity of microbial co-occurrence networks was shaped by several biogeographic parameters, among which temperature was the strongest one.

4. Discussion

Ecological network analysis is an effective method for identifying potential species interactions and co-occurrence patterns that cannot be observed directly (Fath et al., 2007). Multiple methods have now been developed to infer microbial co-occurrence networks using different types of data (Zhou et al., 2010; Faust et al., 2012; Friedman and Alm, 2012; Xia et al., 2013). Although strict statistical methods and cutoffs were used to construct highly confident co-occurrence networks across multiple domains, one potential issue was the possibility of introducing false positives when integrating *nifH* and 16S amplicon data. By checking the connections established between 16S and *nifH* OTUs, no OTUs belonging to the same genera was found. In addition, previous shotgun metagenome sequencing suggested <1% diazotrophic community abundance in soil ecosystems (Tu et al., 2017). Therefore, the chance of getting artificial connections between 16S and *nifH* OTUs would be very low. Although potential false positives might also be introduced due to preferential amplification when using relative abundance data, the importance of relative abundance were not ignored when comparing with occurrence data. Microbial co-occurrence patterns revealed in this study were used to infer potential biogeographic patterns for the complexity of microbial communities beyond conventional species richness and abundance. The study moves beyond conventional descriptive analysis of microbial community diversity and structure, and illustrates how new methodologies can help elucidate potential mechanisms governing microbial community assembly across time and space.

In this study, microbial co-occurrence networks were constructed and analyzed by recruiting 126 samples in six American forests collected in summer, the season that both microorganisms and plants are most active. As a result, we found that tropical and temperate forests differed significantly in microbial co-occurrence network properties. Distinct module hubs were found between tropical and temperate forest networks. Microbial co-occurrence networks in tropical forests were less complex than those in temperate forests in terms of several network properties, such as normalized number of nodes and links, average connectivity, H index of nodes connectivity and betweenness of centrality. Because tropical ecosystems generally harbor much higher biodiversity for both macro-organisms (Gaston, 2000; Willig et al., 2003; Hillebrand, 2004; Mittelbach et al., 2007) and microorganisms (Tu et al., 2016a; Zhou et al., 2016), it is expected that microbial species in tropical rainforests would interact with more species, leading to less specialized biotic interactions in species-rich trophic forests (Schleuning et al., 2012). This striking observation suggests that higher microbial diversity does not necessarily come with more complex co-occurrence networks. From a theoretical perspective, this observation could be explained by at least two ecological theories. The first one is the species-energy relationship (Wright, 1983; Gaston, 2000). Tropical rainforests harbor higher primary productivity and species diversity than temperate forests, providing more diverse energy sources for the microbial communities. As a result, microbial species in tropical ecosystems tend to be supported by more environmental energy and nutrients, instead of by interacting with other species in complex manners. This is also supported by our observation that network parameters such as average connectivity and H-index were significantly negatively correlated with plant richness and diversity. The second one is functional redundancy for microbial communities (Allison and Martiny, 2008; Miki et al., 2014). Higher microbial diversity in tropical ecosystems could lead to higher degree of functional redundancy. Because microbial species tend to interact with each other by function/metabolites preference (Levy and Borenstein, 2013; Tu et al., 2016b), high microbial diversity and functional redundancy in tropical ecosystems provides more chances for microbial species in establishing relationships within neighborhoods. This, as a result, would lead to weakened microbial correlations and simplified co-occurrence networks. Notably, similar trend was also observed for macro-organisms that more restricted and specified niches were found towards lower latitudes, which was also explained by the higher diversity of energy resources at low latitudes (Araújo and Costa-Pereira, 2013).

Keystone nodes were dramatically different between microbial co-occurrence networks in tropical and temperate forests. The module hubs in tropical forest networks were dominated by OTUs belonging to *Proteobacteria*, while the module hubs in temperate forest networks were dominated by *Acidobacteria* OTUs. Because the taxonomic information of most of these keystone OTUs were unknown at species or even genus level, it was almost impossible to confidently infer the potential ecological function of these OTUs. Limited information suggested that these keystone OTUs (e.g. *Syntrophobacterales*, *Burkholderiales*, *Rhizobiales*, *Acidobacteria* Gp1) are mainly aerobic and heterotrophic organic decomposers that can provide nutrients to plants and other organisms in the environment (Boone and Bryant, 1980; Master and Mohn, 1998; Delmotte et al., 2009; Erlacher et al., 2015; Kielak et al., 2016). Interestingly, environmental factors seemed to play important roles for the dominance of different keystone nodes in temperate and tropical networks. For example, the dominance of *Acidobacteria* OTUs as keystone nodes could be due to the lower pH, higher C/N ratio and lower temperature in temperate forests (Jones et al., 2009; Männistö et al., 2013).

Higher frequencies of fungal-microbial and diazotrophic-microbial co-occurrence patterns were observed in tropical rainforest ecosystems. Fungi are ubiquitous in the environment and play important ecological functions associated with nutrient and carbon cycling processes in soil (Christensen, 1989). Soil diazotrophs also play important ecological roles that convert atmospheric N to biologically available ammonium, and contribute about 128 Tg N per year to terrestrial ecosystems (Galloway et al., 2004). Metabolic theory of ecology predicts that higher temperatures in tropical rainforests will drive accelerated rates of ecosystem metabolism, energy flow, and nutrition turnover rate (Brown et al., 2004). These accelerated rates are likely being partly contributed by the higher frequency of fungal-microbial and diazotrophic-microbial interactions, reflecting the metabolic theory of ecology (Brown et al., 2004) at the angle of microbial co-occurrence networks. This is generally consistent with the diversity gradient patterns for macro- and microbial community structure and diversity (Brown et al., 2004; Fuhrman et al., 2008; Fuhrman, 2009; Tu et al., 2016a; Zhou et al., 2016). However, such observation may vary with different soil types and/or seasons. For example, greater saprotrophic fungal activity in the autumn than in the summer was found for temperate forests (Voříšková et al., 2014), which may lead to differed microbial co-occurrence patterns.

Documenting whether microbial co-occurrence networks exhibit any biogeographic pattern is a great challenge in microbial ecology. Unraveling the linkages between microbial co-occurrence networks and biogeography could provide novel insights into the community assembly process of microorganisms (Barberán et al., 2012), and help move the field beyond traditional description of community composition and structure. The current study documents clear biogeographic patterns for microbial co-occurrence networks that temperate forest ecosystems exhibited more complex microbial co-occurrence networks than tropical ecosystems, and microbial co-occurrence networks were mainly modulated by temperature, followed by plant diversity, latitude, soil nitrogen, and precipitation. Our previous studies on these six forests suggested clear temperature and latitudinal gradient patterns of diversity for prokaryotic, fungal and soil diazotrophic communities (Tu et al., 2016a; Zhou et al., 2016). As we expected a close relationship between microbial co-occurrence networks and community diversity, we also hypothesized that microbial co-occurrence networks may follow traditional biogeographic patterns (e.g. latitudinal gradient and pH

gradient), as have been observed for macro- and microbial community diversity and structure (Fierer and Jackson, 2006; Lomolino et al., 2006; Martiny et al., 2006; Green et al., 2008). While we expected that high microbial diversity would lead to high community complexity in tropical forests, the complexity of microbial co-occurrence networks decreased with increasing temperature and decreasing latitude, which is opposite to the traditional latitudinal gradient patterns of biodiversity. The observed biogeographic patterns for microbial co-occurrence network complexity were also quite different from the well-recognized pH gradient patterns for microbial diversity in soil ecosystems (Fierer and Jackson, 2006; Griffiths et al., 2011) that no significant correlation was found between soil properties (e.g. pH and TC) and network complexity parameters. Such inconsistent observation could be due to the relatively narrow range of soil pH, but wide spanning of mean annual temperature in these six forests. This was generally consistent with our previous observations that temperature rather than pH was the strongest factor shaping microbial community diversity and structure in these six forests (Tu et al., 2016a; Zhou et al., 2016).

Interestingly, parameters related with node connectivity (e.g. average connectivity and H index) were mainly correlated with latitudinal parameters (e.g. latitude, temperature, precipitation and plants) and soil ammonia, while the network modularity was mainly correlated with latitudinal parameters (e.g. temperature and precipitation) and soil moisture. As node connectivity reflects the degree that each species co-occurs with other species in the network, the high correlation between soil ammonia and node connectivity parameters suggested the importance of ammonia in potential microbial interactions, which has also been observed for fungal communities (Tu et al., 2015). This suggested the availability of nutrition ammonia could be an important factor driving the interactions among microbial species. As microbial species in the same module could be regarded as sharing similar niches, the high correlation between network modularity and biogeographic properties (e.g. temperature, precipitation and soil moisture) also suggested the importance of these parameters in shaping niche specialization of microbial communities. The observed gradient pattern of network modularity was also similarly observed for ecological niche specialization of macro-organisms (Araújo and Costa-Pereira, 2013). Interestingly, such observation is quite consistent with MacAuthur's latitude–niche breadth hypothesis (MacArthur, 1984).

In summary, this study documented the biogeographic patterns of microbial co-occurrence networks in American forests at the continental scale. Our results showed that temperate forest ecosystems exhibited more complex microbial co-occurrence network patterns than tropical ecosystems, and microbial co-occurrence networks were mainly modulated by temperature, followed by plant diversity, soil N and precipitation. This study suggests that biogeographic variables like temperature not only mediate microbial community diversity and composition, but also the co-occurrence ecological networks among microbial species. Notably, the study was carried out based on soil samples collected at a single time point in summer and with relatively low coverage of soil types, whether the same patterns could be observed in different seasons and in other forests and soil types remain to be further explored. In addition, further experimental validations are also required to verify the potential relationship among different microbial species.

Declaration of competing interest

The authors declare that none of the materials presented in this manuscript has been previously published, nor is under consideration for publication elsewhere. We also certify that there is no conflict of interest with all authors regarding the manuscript.

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Appendix A. Supplementary data

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References

Allison, S.D., Martiny, J.B., 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences 105, 11512–11519.
Araújo, M.S., Costa-Pereira, R., 2013. Latitudinal gradients in intraspecific ecological diversity. Biology Letters 9, 20130778.
Barberán, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. The ISME Journal 6, 343–351.
Boone, D.R., Bryant, M.P., 1980. Propionate-degrading bacterium, syntrophobacter wolinii sp. nov. Gen. Nov., from methanogenic ecosystems. Applied and Environmental Microbiology 40, 626–632.
Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 85, 1771–1789.
Buckley, D.H., Schmidt, T.M., 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environmental Microbiology 5, 441–452.
Butts, C.T., 2008. Social network analysis with sna. Journal of Statistical Software 24, 1–51.
Cho, J.C., Tiedje, J.M., 2000. Biogeography and degree of endemicity of fluorescent Pseudomonas strains in soil. Applied and Environmental Microbiology 66, 5448–5456.
Christensen, M., 1989. A view of fungal ecology. Mycologia 81, 1–19.
Csardi, G., Nepusz, T., 2006. The igraph software package for complex network research. InterJournal Complex Systems 1695, 1–9.
Cumming, G.S., Bodin, Ö., Ernstson, H., Elmqvist, T., 2010. Network analysis in conservation biogeography: challenges and opportunities. Diversity and Distributions 16, 414–425.
Delmotte, N., Knief, C., Chaffron, S., Innerebner, G., Roschitzki, B., Schlapbach, R., von Mering, C., Vorholt, J.A., 2009. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. Proceedings of the National Academy of Sciences 106, 16428–16433.
Deng, Y., Jiang, Y.-H., Yang, Y., He, Z., Luo, F., Zhou, J., 2012. Molecular ecological network analyses. BMC Bioinformatics 13, 1.
Deng, Y., Zhang, P., Qin, Y., Tu, Q., Yang, Y., He, Z., Schadt, C.W., Zhou, J., 2016. Network succession reveals the importance of competition in response to emulsified vegetable oil amendment for uranium bioremediation. Environmental Microbiology 18, 205–218.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10, 996.
Erlacher, A., Cernava, T., Cardinale, M., Soh, J., Sensen, C.W., Grube, M., Berg, G., 2015. Rhizobiales as functional and endosymbiontic members in the lichen symbiosis of Lobaria pulmonaria L. Frontiers in Microbiology 6, 53-53.
Fath, B.D., Scharler, U.M., Ulanowicz, R.E., Hannon, B., 2007. Ecological network analysis: network construction. Ecological Modelling 208, 49–55.
Faust, K., Sathirapongsasuti, J.F., Izard, J., Segata, N., Gevers, D., Raes, J., Huttenhower, C., 2012. Microbial co-occurrence relationships in the human microbiome. PLoS Computational Biology 8, e1002606.
Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences 103, 626–631.
Friedman, J., Alm, E.J., 2012. Inferring correlation networks from genomic survey data. PLoS Computational Biology 8, e1002687.
Fuhrman, J.A., 2009. Microbial community structure and its functional implications. Nature 459, 193–199.
Fuhrman, J.A., Steele, J.A., Hewson, I., Schwalbach, M.S., Brown, M.V., Green, J.L., Brown, J.H., 2008. A latitudinal diversity gradient in planktonic marine bacteria. Proceedings of the National Academy of Sciences 105, 7774–7778.
Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S. P., Asner, G.P., Cleveland, C., Green, P., Holland, E., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153–226.
Gaston, K.J., 2000. Global patterns in biodiversity. Nature 405, 220–227.
Green, J.L., Bohannan, B.J., Whitaker, R.J., 2008. Microbial biogeography: from taxonomy to traits. Science 320, 1039–1043.
Griffiths, R.I., Thomson, B.C., James, P., Bell, T., Bailey, M., Whiteley, A.S., 2011. The bacterial biogeography of British soils. Environmental Microbiology 13, 1642–1654.
Guimerà, R., Nunes Amaral, L.A., 2005. Functional cartography of complex metabolic networks. Nature 433, 895.

Hillebrand, H., 2004. On the generality of the latitudinal diversity gradient. The American Naturalist 163, 192–211.
Hirsch, J.E., 2005. An index to quantify an individual's scientific research output. Proceedings of the National Academy of Sciences 16569–16572.
Jones, R.T., Robeson, M.S., Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. The ISME Journal 3, 442–453.
Kielak, A.M., Barreto, C.C., Kowalchuk, G.A., van Veen, J.A., Kuramae, E.E., 2016. The ecology of Acidobacteria: moving beyond genes and genomes. Frontiers in Microbiology 7, 744-744.
Konstantinidis, K.T., Tiedje, J.M., 2005. Genomic insights that advance the species definition for prokaryotes. Proceedings of the National Academy of Sciences 102, 2567–2572.
Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., Jones, S.J., Marra, M.A., 2009. Circos: an information aesthetic for comparative genomics. Genome Research 19, 1639–1645.
Levy, R., Borenstein, E., 2013. Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules. Proceedings of the National Academy of Sciences 110, 12804–12809.
Lomolino, M.V., Riddle, B.R., Brown, J.H., Brown, J.H., 2006. Biogeography. Sinauer Associates Sunderland, MA.
Lu, L., Zhou, T., Zhang, Q.-M., Stanley, H.E., 2016. The H-index of a network node and its relation to degree and coreness. Nature Communications 7.
Ma, B., Wang, H., Dsouza, M., Lou, J., He, Y., Dai, Z., Brookes, P.C., Xu, J., Gilbert, J.A., 2016. Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. The ISME Journal 10, 1891–1901.
MacArthur, R.H., 1984. Geographical Ecology: Patterns in the Distribution of Species. Princeton University Press.
Männistö, M.K., Kurhela, E., Tiirola, M., Häggblom, M.M., 2013. Acidobacteria dominate the active bacterial communities of Arctic tundra with widely divergent winter-time snow accumulation and soil temperatures. FEMS Microbiology Ecology 84, 47–59.
Martín González, A.M., Dalsgaard, B., Nogués-Bravo, D., Graham, C.H., Schleuning, M., Maruyama, P.K., Abrahamczyk, S., Alarcón, R., Araujo, A.C., Araújo, F.P., de Azevedo Jr., S.M., Baquero, A.C., Cotton, P.A., Ingversen, T.T., Kohler, G., Lara, C., Las-Casas, F.M.G., Machado, A.O., Machado, C.G., Maglianesi, M.A., McGuire, J.A., Moura, A.C., Oliveira, G.M., Oliveira, P.E., Ornelas, J.F., Rodrigues, L.d.C., Rosero-Lasprilla, L., Rui, A.M., Sazima, M., Timmermann, A., Varassin, I.G., Vizentin-Bugoni, J., Wang, Z., Watts, S., Rahbek, C., Martinez, N.D., 2015. The macroecology of phylogenetically structured hummingbird–plant networks. Global Ecology and Biogeography 24, 1212–1224.
Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J. L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Ovreas, L., Reysenbach, A.-L., Smith, V.H., Staley, J.T., 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology 4, 102–112.
Master, E.R., Mohn, W.W., 1998. Psychrotolerant bacteria isolated from arctic soil that degrade polychlorinated biphenyls at low temperatures. Applied and Environmental Microbiology 64, 4823–4829.
McArthur, J.V., Kovacic, D.A., Smith, M.H., 1988. Genetic diversity in natural populations of a soil bacterium across a landscape gradient. Proceedings of the National Academy of Sciences 85, 9621–9624.
Miki, T., Yokokawa, T., Matsui, K., 2014. Biodiversity and multifunctionality in a microbial community: a novel theoretical approach to quantify functional redundancy. Proceedings of the Royal Society B: Biological Sciences 281.
Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Brown, J.M., Bush, M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N., Lessios, H.A., 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecology Letters 10, 315–331.
Newman, M.E., 2006. Modularity and community structure in networks. Proceedings of the National Academy of Sciences 103, 8577–8582.
Oldham, M.C., Konopka, G., Iwamoto, K., Langfelder, P., Kato, T., Horvath, S., Geschwind, D.H., 2008. Functional organization of the transcriptome in human brain. Nature Neuroscience 11, 1271–1282.
Olesen, J.M., Bascompte, J., Dupont, Y.L., Jordano, P., 2007. The modularity of pollination networks. Proceedings of the National Academy of Sciences 104, 19891–19896.
Pellissier, L., Albouy, C., Bascompte, J., Farwig, N., Graham, C., Loreau, M., Maglianesi, M.A., Melián, C.J., Pitteloud, C., Roslin, T., Rohr, R., Saavedra, S., Thuiller, W., Woodward, G., Zimmermann, N.E., Gravel, D., 2018. Comparing species interaction networks along environmental gradients. Biological Reviews 93, 785–800.
Poisot, T., Canard, E., Mouillot, D., Mouquet, N., Gravel, D., 2012. The dissimilarity of species interaction networks. Ecology Letters 15, 1353–1361.
Schleuning, M., Fründ, J., Klein, A.-M., Abrahamczyk, S., Alarcón, R., Albrecht, M., Andersson, Georg K.S., Bazarian, S., Böhning-Gaese, K., Bommarco, R., Dalsgaard, B., Dehling, D.M., Gotlieb, A., Hagen, M., Hickler, T., Holzschuh, A., Kaiser-Bunbury, Christopher N., Kreft, H., Morris, Rebecca J., Sandel, B., Sutherland, William J., Svenning, J.-C., Tscharntke, T., Watts, S., Weiner, Christiane N., Werner, M., Williams, Neal M., Winqvist, C., Dormann, Carsten F., Blüthgen, N., 2012. Specialization of mutualistic interaction networks decreases toward tropical latitudes. Current Biology 22, 1925–1931.
Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Research 13, 2498–2504.
Shay, P.-E., Winder, R.S., Trofymow, J.A., 2015. Nutrient-cycling microbes in coastal Douglas-fir forests: regional-scale correlation between communities, in situ climate, and other factors. Frontiers in Microbiology 6.
Staddon, W.J., Trevors, J.T., Duchesne, L.C., Colombo, C.a., 1998. Soil microbial diversity and community structure across a climatic gradient in western Canada. Biodiversity & Conservation 7, 1081–1092.
Tu, Q., Deng, Y., Yan, Q., Shen, L., Lin, L., He, Z., Wu, L., Van Nostrand, J.D., Buzzard, V., Michaletz, S.T., 2016a. Biogeographic patterns of soil diazotrophic communities across six forests in the North America. Molecular Ecology 25, 2937–2948.
Tu, Q., He, Z., Wu, L., Xue, K., Xie, G., Chain, P., Reich, P.B., Hobbie, S.E., Zhou, J., 2017. Metagenomic reconstruction of nitrogen cycling pathways in a CO2-enriched grassland ecosystem. Soil Biology and Biochemistry 106, 99–108.
Tu, Q., Yuan, M., He, Z., Deng, Y., Xue, K., Wu, L., Hobbie, S.E., Reich, P.B., Zhou, J., 2015. Fungal communities respond to long-term CO_2 elevation by community reassembly. Applied and Environmental Microbiology 81, 2445–2454.
Tu, Q., Zhou, X., He, Z., Xue, K., Wu, L., Reich, P., Hobbie, S., Zhou, J., 2016b. The diversity and Co-occurrence patterns of N_2-fixing communities in a CO_2-enriched grassland ecosystem. Microbial Ecology 71, 604–615.
Voříšková, J., Brabcová, V., Cajthaml, T., Baldrian, P., 2014. Seasonal dynamics of fungal communities in a temperate oak forest soil. New Phytologist 201, 269–278.
Wagg, C., Bender, S.F., Widmer, F., van der Heijden, M.G.A., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences 111, 5266–5270.
Weiser, M.D., Michaletz, S.T., Buzzard, V., Deng, Y., He, Z., Shen, L., Enquist, B.J., Waide, R.B., Zhou, J., Kaspari, M., 2018. Toward a theory for diversity gradients: the abundance–adaptation hypothesis. Ecography 41, 255–264.
Weiss, S., Van Treuren, W., Lozupone, C., Faust, K., Friedman, J., Deng, Y., Xia, L.C., Xu, Z.Z., Ursell, L., Alm, E.J., Birmingham, A., Cram, J.A., Fuhrman, J.A., Raes, J., Sun, F., Zhou, J., Knight, R., 2016. Correlation detection strategies in microbial data sets vary widely in sensitivity and precision. The ISME Journal 10, 1669–1681.
Willig, M.R., Kaufman, D.M., Stevens, R.D., 2003. Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. Annual Review of Ecology, Evolution and Systematics 273–309.
Wright, D.H., 1983. Species-energy theory: an extension of species-area theory. Oikos 41, 496–506.
Xia, L.C., Ai, D., Cram, J., Fuhrman, J.A., Sun, F., 2013. Efficient statistical significance approximation for local similarity analysis of high-throughput time series data. Bioinformatics 29, 230–237.
Zavaleta, E.S., Pasari, J.R., Hulvey, K.B., Tilman, G.D., 2010. Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. Proceedings of the National Academy of Sciences 107, 1443–1446.
Zhou, J., Bruns, M.A., Tiedje, J.M., 1996. DNA recovery from soils of diverse composition. Applied and Environmental Microbiology 62, 316–322.
Zhou, J., Deng, Y., Luo, F., He, Z., Tu, Q., Zhi, X., 2010. Functional molecular ecological networks. mBio 1 e00169-00110.
Zhou, J., Deng, Y., Luo, F., He, Z., Yang, Y., 2011. Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO2. mBio 2 e00122-00111.
Zhou, J., Deng, Y., Shen, L., Wen, C., Yan, Q., Ning, D., Qin, Y., Xue, K., Wu, L., He, Z., 2016. Temperature mediates continental-scale diversity of microbes in forest soils. Nature Communications 7, 12083.