Title

A latitudinal pattern of plant leaf-associated bacterial community assembly

Authors

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Abstract

Plant-associated microbes are essential for promoting plant well-being, maintaining biodiversity, and supporting ecosystem function. However, little is known about the geographic distribution of plant-microbe symbioses and how they are formed and change along latitudinal gradients. Here we identified leaf bacteria for 328 plant species sampled from 10 forests along a tropical to temperate gradient in China. We analyzed the diversity and composition of plant leaf-associated bacteria and quantified the contributions of hosts, habitats, and neighborhood plants to the plant-bacterial symbiosis. We found a strong latitudinal gradient in leaf bacterial diversity and composition. Bacterial assemblages on leaves were most strongly selected by host plants, and the selection pressure increased with latitude. In contrast, at low latitudes and at large geographical scales multiple factors were found to jointly regulate bacterial community composition. Our result also showed that plant-bacteria symbiotic networks were structured by network hub bacteria taxa with high co-occurrence network centrality, and the abundance of temperate hub taxa was more influenced by host plants than that in tropical forests. For the first time, we documented a previously unrecognized latitudinal gradient in plant-bacterial symbioses that was regulated by a joint effect of multiple factors at low latitudes but mostly by host selection at high latitudes, implying that leaf microbiomes are likely to respond differently to global change along the latitudinal gradient.

Main text

All animals and plants are colonized by microorganisms, which are critical for ecosystem function and global biogeochemical cycles[1]. As one of the most common types of biotic interactions, plant-microbial symbioses are essential for plant growth, health and stress resilience, and also for supporting ecosystem functions including enhancing productivity, biogeochemical cycling, and diversity maintenance[2,3]. However, our knowledge about plant-microbial symbioses is highly variable among symbiosis types, with belowground symbioses such as nitrogen-fixation symbioses and root mycorrhizal symbioses being extremely well studied while aboveground plant-microbe symbioses have not been explored as deeply[4,5]. An example of aboveground symbioses is microbial assemblages on leaves – a metasymbiosis that is formed not by individual microbes or a specific group of microbes (i.e. *Bradyrhizobium* bacteria or glomeromycete mycorrhizal fungi in the case of roots) but by the diverse metacommunity of microorganisms available to colonize leaves[6]. How such leaf-microbe associations are formed and maintained is a critical but unanswered question, leading to a major concern that the future role of plant microbiomes in supporting plant well-being and sustaining ecosystem functioning may be compromised in the face of global change[7,8]. To address this question, it is necessary to understand the geographic variation of the phyllosphere metasymbiosis across a broad geographic scale in order to gain insights into how leaf-microbe associations are assembled and how they vary across different habitat conditions and host compositions. This understanding of geographic patterns of phyllosphere microbial diversity is indispensable for predicting the responses of plant microbiomes to global change[7,8].

Bacteria are the most abundant microbes in the phyllosphere with an estimated 10^7 bacterial cells per cm^2 of leaf surface[9]. Considering the total area of plant leaf surfaces on Earth,

the global bacterial population in the phyllosphere is enormous (up to 10^{26} cells), as is their impact on plant communities and ecosystem functions through effects including altering plant immunity, modifying plant hormone production and facilitating global carbon and nitrogen cycling[10–12]. The assembly of this ubiquitous bacterial leaf symbiosis is governed by a range of factors that influence the community assembly processes such as selection and dispersal[13]. For example, host plant attributes including species identity, functional traits and phylogeny shape bacterial community assembly through selection processes[14–16]. Neighborhood plants also select for and create a bacterial metacommunity that alters the composition of local communities through dispersal[17,18]. Additionally, abiotic environments such as temperature and microhabitats which are often spatially autocorrelated have been observed to affect leaf bacterial diversity[19–21]. However, these drivers of leaf bacterial assembly have commonly been examined separately in previous studies. As a result, there is poor understanding of the relative importance of these different drivers of plant-bacterial symbioses. Furthermore, current knowledge on the drivers of plant-microbe associations is almost exclusively based on individual sites; we understand very little how assembly processes of bacteria community vary along latitudinal gradients, in stark contrast with our knowledge about latitudinal diversity gradients of higher plants and animals[22,23].

Studies of microbial co-occurrence networks have revealed hub taxa that occupy central positions within microbial interaction networks, and have demonstrated that these hub taxa play key roles driving microbial community structure and function[24,25]. At the metacommunity level, hub taxa connect local communities via dispersal and gene flow and influence local community assembly by microbe-microbe and plant-microbe interactions, through which their population dynamic could impact large-scale patterns of plant-bacterial interactions[26–28]. Identifying the hub

98 taxa in plant-bacterial association at the metacommunity level is a pressing need given their
99 importance in mediating eco-evolutionary feedbacks, but has been limited to date by a lack of
100 large-scale datasets on plant-microbe associations[29]. Furthermore, knowledge about the factors
101 that control the geographic distribution of these hub bacterial taxa is essential for predicting
102 future change in microbiome structure due to gain or loss of hub taxa, but is currently
103 unavailable for plant-associated microbes[30].

104 Here, we addressed these knowledge gaps by investigating geographic patterns of
105 phyllosphere bacterial diversity, their network structure and assembly mechanisms along a large
106 latitudinal gradient for a network of stem-mapped forest sites across China. We quantified
107 bacterial communities using high-throughput sequencing of the 16S rRNA gene for a total of
108 1453 leaf samples from 328 plant species from 10 forest sites across major forest biomes in
109 China. Specifically, we aimed to (1) document latitudinal patterns of leaf-associated bacterial
110 diversity, (2) disentangle the effects of hosts, environments, neighborhood plants and spatial
111 factors on the community assembly of phyllosphere bacteria along the latitudinal gradient and
112 across geographic scales, and (3) construct plant-bacterial symbiosis networks and identify the
113 metacommunity-level hub taxa that are instrumental to plant-bacteria associations; we further
114 modeled the distribution of these bacterial hub taxa along the latitudinal gradient.

115 **Results**

116 We identified 49254 16S rRNA gene amplicon sequence variants (ASV) from 1453 leaf
117 samples, belonging to 237 bacterial orders and 386 families. *Proteobacteria* were the dominant
118 phylum, accounting for 70% of the total abundance of sequences across all samples.
119 *Beijerinckiaceae* (*Rhizobiales*) were most abundant in tropical and subtropical regions while
120 *Sphingomonadaceae* (*Sphingomonadales*) were most abundant in temperate forests (Fig. S1).

ASV richness of phyllosphere bacteria decreased with latitude (Fig. 1a). There was strong evidence that ASV richness was correlated positively with the diversity of neighborhood plants, negatively with slope, and was linked with elevation and annual temperature in quadratic relationships (Fig. S2). We used global non-metric multidimensional scaling ordinations to visualize the variation in bacterial community composition and show clear clustering of bacterial communities by study sites and host taxonomy (Fig. 1b&S3). Permutational multivariate analysis of variance revealed strong evidence that climate, soil conditions, topography, neighborhood plant diversity and host traits all impact bacterial community assembly (Table S1). There was a higher similarity of phyllosphere bacterial communities on phylogenetically closely related plants and spatially closer samples, leading to host phylogenetic structure and spatial autocorrelation effects on bacterial community assembly at both local and large scales (Fig. S4).

We quantified the variation in bacterial ASV richness and composition explained by multiple variables that have been proposed as drivers of host-associated microbial diversity using generalized linear models and redundancy analysis respectively. The variables included host-related factors (phylogenetic eigenvectors + plant traits), abiotic environment (soil conditions + topography + climate), diversity and abundance of neighborhood plants, and spatial eigenvectors (see Methods). At the local scale of individual sites, these models explained 26%-76% of the variation in ASV richness and 19%-36% of the variation in community composition of phyllosphere communities. At regional levels, these models explained 27%-68% of the variation in species richness, and 33%-42% of the variation in community composition respectively. At a 'global' scale (defined as the scale including all study sites), the model explained 43% of the total variation in phyllosphere bacterial species richness and 47% of the variation in community composition across all sites (Fig. 2&S5).

We used variation partitioning to disentangle the independent and joint effect of these variables and quantify their relative importance in structuring bacterial diversity and community assembly. Generally, the independent effect of host was the most important in explaining both phyllosphere bacterial ASV richness and community composition, accounting for 15-89% of the explained variance, followed by abiotic environments and spatial eigenvectors (contributions of up to 29% and 25% respectively), while the attributes of plant neighborhoods were less important (only contributing 9% of explained variance). There was a strong joint effect of different variables, which accounted for 8-72% of the explained variation in bacterial community composition (Fig. 2). The relative importance of these factors varied across geographic scales and was highly latitude dependent; for example, the independent effect of host was positively correlated with latitude and was less important at large scale, while the joint effect of these four variables decreased with latitude and was more pronounced at large scales, and the overall contribution of host-related factors increased while that of other factors such as environment decreased with latitude (Fig. 3).

We constructed metacommunity-level networks of plant-bacterial associations at regional and global scales and quantified the topological positions of ASVs within each co-occurrence network. Hub ASVs with high network betweenness centrality were identified for tropical, subtropical, and temperate regional networks and a global network respectively (see Methods). Hub bacterial ASVs differed across regions; in subtropical and tropical regions, bacteria hubs were primarily ASVs belonging to the *Rhizobiales* genera *1174-901-12, Methylocella* and *Methylobacterium*, while in temperate networks the hubs included *Sphingomonas* (*Sphingomonadales*) and *Hymenobacter* (*Cytophagales*) (Fig. S6). These bacterial ASVs connect with most of the nodes (i.e. plant species) in the association networks and structure plant-

167 bacterial association networks at metacommunity levels; plant-phyllosphere bacteria association
168 networks at local sites were interconnected by hub bacterial ASVs to form regional-scale
169 networks, and these regional networks were further linked together through hub ASVs at global
170 scales (Fig. 4).

171 The distribution of phyllosphere bacterial hubs was clustered along latitudinal gradients
172 (Fig. 1c). To determine the relative importance of different factors structuring the biogeography
173 of these hub ASVs, we applied a joint species distribution modeling approach where nine habitat
174 variables were taken as fixed effects and two distance matrices representing the spatial and host
175 phylogenetic structure as random effects (see Methods). The models explained up to 90% and
176 73% of the variation in presence-absence and abundance of hub bacterial ASV, respectively.
177 Overall, climate (including temperature, precipitation and isothermality) was the most important
178 factor explaining the distribution of phyllosphere bacterial hub ASVs (Fig. 5&S7). There was
179 also a major contribution of the random effects of spatial factors and host phylogeny on hub
180 ASV abundance, highlighting the importance of taking spatial and host phylogenetic structure
181 into account for unbiased inference and to improve the prediction of the distribution of plant
182 microbiomes. Host plant attributes (leaf traits and phylogeny) were more important in
183 determining the abundance of hub ASVs in temperate regions, while spatial-related effects were
184 more important in influencing tropical hub ASVs (Fig. 6). Additionally, we found a strong
185 phylogenetic signal in the responses of ASVs to habitat covariance, indicating that
186 phylogenetically closely related ASVs have similar habitat preferences (Fig. S8).

187 **Discussion**

188 In this study, we revealed for the first time a strong latitudinal pattern in the diversity,
189 community composition and distribution of plant-leaf associated bacteria, highlighting the

varying strength of community assembly processes along the latitudinal gradient in shaping plant leaf-microbial symbioses. It is remarkable that we found a shift from strong host filtering in bacterial community assembly in temperate forests, to weak host filtering but a strong joint effect of multiple drivers in the tropics. These results suggest that plant-associated bacteria on leaves are formed following different rules across latitudes, with an increased complexity of different processes acting to explain bacterial symbioses at lower latitudes. The latitude-dependent community assembly processes were consistently evident in driving bacterial ASV richness, community composition and the abundance of metacommunity-level hub bacteria taxa, which together provide the most comprehensive understanding to date of the patterns and mechanisms of plant-bacterial associations at large geographical scales. Our findings imply responses of plant-bacteria symbioses to host plant composition and environmental change differ in tropical vs. temperate regions, serving as a baseline for predicting and managing plant-microbe symbioses under global change.

Although host attributes, environment, climate, plant neighborhoods and spatial factors have been shown to influence the diversity of phyllosphere bacteria in previous studies [14,16,18–20], most of these factors were analyzed individually in these studies. As such, their relative importance was unknown, which made it difficult to infer the processes underlying the observed host-microbe associations[31]. By simultaneously evaluating a broad range of factors across spatial scales, we have shown that host-related factors including plant phylogeny and leaf traits were the most important drivers of phyllosphere bacterial community assembly, especially in temperate regions. This result suggested that phylogenetically conserved host attributes including specific leaf area and leaf nutrient concentrations could select for leaf-associated bacteria[14,15]. Our results of independent effects of hosts on bacterial diversity at large spatial scales indicated consistent

matching between host plant and phyllosphere bacteria regardless of climates, which represents a potential consequence of coevolutionary feedbacks between plant hosts and their associated microbes[15,32]. The importance of host plant selection mediated by phylogenetically conserved traits could further lead to host specialization of plant-associated microbes, which may allow microbes to coexist and explain their incredible diversity on leaves, and also contribute to the host-specific effects of plant-associated microbes in structuring plant communities[33–35].

While host selection was among the most important factor in explaining phyllosphere bacterial community assembly, the joint effect of multiple other factors also played a critical role particularly in tropical forests. Many of these factors including spatial structure and neighboring plant communities are likely related to the importance of stochastic processes including dispersal for community assembly of plant-associated bacteria, suggesting a latitudinal patterns in the relative contribution of deterministic and stochastic process to shaping plant-bacteria association. This result concurred with the prediction of the niche-neutral continuum hypothesis that the importance of niche-based processes such as selection are stronger in low diversity temperate regions while the importance of species-neutral processes such as dispersal are more important in high diversity tropical regions [22,36]. Previous studies have shown that the processes structuring plant community assembly increasingly deviated from neutrality with latitude, and here we provided the first evidence that this hypothesis was supported for microbes, suggesting that ecological hypotheses developed based on evidence from macroorganisms also apply to explain differences in microbial community assembly along latitudinal gradients[22].

Although the composition of phyllosphere bacterial communities varied greatly across study sites along the latitudinal gradient, the different bacterial communities were interconnected by a small proportion of hub bacterial ASVs to form large-scale plant-bacterial cooccurrence

236 networks. These hub ASVs were associated with a wide range of plant species in a broad
237 geographic space and include taxa such as *Methylorubrum* and *Sphingomonas* that have been
238 shown to play key roles in promoting plant health and productivity[10]. Additionally, the hub
239 bacterial taxa at the metacommunity level could affect large-scale plant-bacteria interactions: the
240 movement of hub taxa across local communities could mediate otherwise discrete ecological
241 processes within a metacommunity. For example, although plant species differing in growth-
242 mortality strategies hosted distinct leaf bacteria communities, the bacteria on one plant species
243 can affect that of other plant species through their influence on the metacommunity of shared
244 hub bacteria taxa[14,26]. Likewise, hub taxa could synchronize coevolutionary processes and
245 control the pace of host-bacteria coevolution at metacommunity scales through gene flow[37].
246 Thus, the spatial distribution of hub bacteria taxa should have a major impact on large-scale
247 patterns of plant-bacteria associations[26]. The distribution of hub bacterial ASVs was clustered
248 along the latitudinal gradient and was mainly controlled by climate, host attributes and spatial
249 factors. This suggests that climate change and plant species range shifts could change plant-
250 bacteria interactions through affecting the distribution of those hub taxa. Moreover, the influence
251 of host plant attributes on bacterial abundance was more important for hub taxa in temperate than
252 that in tropical forests, while spatial-related factors were more important for tropical than
253 temperate hub taxa, consistent with the observation of varying strengths of different processes
254 structuring bacterial diversity and community assembly along the latitudinal gradient. This result
255 emphasizes the importance of host selection in temperate regions and dispersal in the tropics to
256 explain the spatial distribution of hub bacteria taxa.
257 Understanding the response of plant microbiomes to global change and the consequent
258 impact on plant communities and ecosystem function requires large-scale studies on the diversity

and biogeography of plant-associated microbes[7,30,38]. Our results show for the first time that the community assembly of plant leaf-associated bacterial communities is driven by processes whose importance varies along a latitudinal gradient. A corollary of this finding is that global change is likely to have different effects on plant leaf-microbe associations along the latitudinal gradient, with tropical associations being more sensitive to climate warming and environmental changes and temperate associations being more sensitive to changes in host plant abundance and plant species range shifts. The direction of the global change effect could even reverse from negative to positive from low latitudes to high latitudes, as recently shown for plant soil microbes[39,40]. Host selection could result in distinct microbiome compositions that exert plant species-specific impacts on host fitness. Although our study showed varying strength of host selection across leaf microbiomes from temperate to tropical forests, it remains to be understood whether the impact of plant leaf microbiomes on plant communities differs along latitude gradients. Additional broad-scale comparative studies that take multiple factors into account will be required to reveal the role of plant microbiomes in mediating the impact of global change on plant communities and ecosystem function.

Methods

Study sites

We conducted the study at 10 forest sites across China with latitude ranging from 18.7°N to 51.8°N (Table S2). The sites are part of a global network of stem-mapped forest plots of the Forest Global Earth Observatory (ForestGEO http://forestgeo.si.edu/). For each site, we selected approximately 1/3 of the tree species and around 3 individuals per species. The choice of these species considered both shade tolerant and intolerant, early- and late-successional species, in

282 order to have a gradient in host attributes that may explain the variation in bacterial community
283 assemblages. In total, 1453 trees from 328 plant species of 148 genera and 59 families were
284 sampled.

285 *Sampling and processing*

286 For each tree individual, 50-100g leaves from the sub-canopy (2-10 m above ground)
287 were collected. Leaves were placed in a sterile plastic bag and washed using 100 mL of a 1:50
288 dilution of Redford buffer (1M Tris, 500 mM EDTA, 1.2% CTAB) within 24 hours after
289 sampling[14]. The suspension was collected and centrifuged at 3300 g for 25 minutes and the pellet
290 was added into an extraction tube to store in -20 °C until DNA extraction. Microbial DNA was
291 extracted following the manufacturer's protocol (QIAGEN Powersoil DNA extraction kit) and
292 the V5-V6 region of bacteria 16S rRNA genes was amplified using primers 799F and 1115R to
293 determine the composition of the phyllosphere bacterial community. PCR products were
294 normalized using a SequalPrep Normalization kit (Thermo Fisher Scientific), pooled and then
295 purified using AMPure to remove contaminants. The DNA library was prepared by mixing
296 equimolar concentrations of DNA from each sample, and then sequenced using Illumina MiSeq
297 Reagent Kit v3 for 300 bp paired-end sequencing.

298 *Bioinformatics*

299 We used DADA2 to identify the amplicon sequence variants (ASV) observed in each
300 sample[41]. The base quality scores (Q) were calculated, and the nucleotides at 19-240 bp and 16-
301 240 bp positions were kept for forward and reverse DNA sequences respectively. Then we
302 inferred error rates and inferred ASVs using DADA2 default parameters[41]. Paired read ends were
303 merged with a minimum overlap of 20. Non-target sequences and chimeras were removed.
304 Taxonomy was assigned to each ASV by comparison with the SILVA SSU r138 database.

We revealed around 116 million high-quality 16S rRNA gene sequences and identified 55693 amplicon sequence variants (ASVs) from 1453 samples. Sequence abundance per sample ranged from 15485 to 522574, which could bias estimates of ASV diversity (Fig. S9a). Thus, we randomly rarefied the ASV table to 15000 sequences per sample using the R package 'vegan', and 49254 ASVs were retained after rarefaction. This eliminated only a small proportion of extremely rare ASVs and had little impact on beta diversity (e.g. community dissimilarity; Fig. S9b). Most ASVs (82%) were identified to the family level, 58% were classified to genus and only 4% were identified to the species level.

Metadata

We collected metadata describing host plant attributes, abiotic environments, neighborhood plants and spatial location. Plant phylogenetic tree was derived from a phylogeny database for vascular plants according to the species identity using the *V.phyloMaker* function in R [42]. Host plant identity and functional traits and abiotic environments including topographic and edaphic variables were derived from the base data of each site. Climate variables including annual mean temperature, annual precipitation, and precipitation seasonality were collected from WorldClim database based on the geographical coordinates of sites. We calculated the richness, abundance, mean phylogenetic distance and mean nearest neighbor distance of neighborhood plants present within 10 meters around the sample, and included the local abundance of host plant species in the metadata (Table S3).

Statistical analysis

We used R (version 3.6) for all statistical analyses. The diversity of phyllosphere bacteria was modelled as a function of variables of host, environment, neighborhood plants and spatial factors. To prepare these variables, Moran's Eigenvector Maps (MEM) was used to decompose

328 geographic distance matrices into a series of orthogonal eigenvectors representing the spatial
329 relationships among samples, and principal coordinates of neighbor matrices (PCNM) was
330 applied on the phylogenetic distance matrix among host plants to generate a series of variables
331 that representing the position of host plant in a multi-dimensional phylogenetical space. Both
332 phylogenetic and spatial eigenvectors can be used in regression to take phylogenetic/spatial
333 autocorrelation into account [43]. Environment, host traits and diversity of plant neighborhoods
334 variables were rescaled using the formula $(x - x_{min})/(x_{max} - x_{min})$ to standardize the effects of the
335 variables. Pairwise Pearson's correlation coefficients were calculated to test the correlation
336 among all variables, and for predictors with correlation coefficient > 0.9, one predictor was
337 randomly removed to reduce multicollinearity.

338 Species richness was calculated as the number of ASVs with abundance > 1, which
339 follows a negative binomial distribution (NBD). To model the effects of variables on richness,
340 we used generalized linear model with NBD as the error structure, implemented with function
341 *glm.nb* in the R package MASS. We first built a candidate set of generalized linear models to
342 represent the individual and joint linear contributions for types of mechanisms (host,
343 environment, neighborhoods and spatial proximity) using the predictors for each mechanism,
344 resulting in a total of 15 models. For each model, a stepwise selection procedure was used for
345 model selection based on Akaike's information criterion corrected for small sample size (AICc).
346 Non-significant variables ($P < 0.05$) were further excluded using the *update* function. Variance
347 inflation factor (VIF) was calculated and variables with VIF > 5 were also removed to avoid
348 multicollinearity. To quantify the individual effects of four types of variables and their
349 interactions on bacterial richness, we used variation partitioning where the independent effect of
350 each factor was evaluated as the difference in R^2 between models with and without this factor,

and the interactive effect was calculated by subtracting the independent effect from overall effects. We used a subset of samples to evaluate the effect of host plant traits as the trait data are available for most but not all samples (Table S3). The effect of plant traits was highly corrected with that of plant phylogeny (Fig. S10). Thus, we only included plant phylogenetic eigenvectors as host variables in the variation partitioning analysis.

We repeated the same variance partition analysis on bacterial composition. To do that, the ASV table was Hellinger transformed to represent the community composition of bacteria. We used redundancy analysis (RDA) to model the effect of those variables on community composition. Variable selection was done for each model using forward selection implemented with the function *forward.sel* in R package packfor following the recommended stopping rules, and variation partitioning was implemented by *varpart* function in the 'vegan' package [44].

The analyses of richness and community composition were conducted at local scales for the 10 sites, at regional scales for tropical, subtropical and temperate forests, and at the global scale by combining all the sites. Specifically, Heishiding (HSD) and Dinghushan (DHS) sites (located at 23.27°N, 23.17°N respectively) were on the northern boundary of the tropics. Although classified as subtropical sites, the two forests are more similar in vegetation composition and climate conditions with the tropical forest Jianfengling (JFL, 18.73°N) than those typical subtropical forests (Table S6). We thus included HSD and DHS as 'tropical' sites, resulting in 3 tropical, 4 subtropical and 3 temperate sites. The relative importance of each factor and their interactive effects were evaluated as the proportion of explained variance, which was further related to latitude using a linear regression.

To construct networks that represent plant-bacteria associations at different geographical scales, we first transformed ASV abundances to presence-absence with a threshold of 15 reads

per sample (0.1% of the total read count of each sample) to be considered present, in order to minimize the effects of extreme rare species and sequencing errors on network construction. The occurrence of ASV in samples was then converted to occurrences on plant species according to the plant host identity of each sample, where a cell entry represented the number of leaf samples that were observed to have the ASV occurring on a plant species. In this step, the same plant species from different sites were represented in different rows as we were interested in how metacommunity bacteria interconnect local plant-bacteria networks across sites. The species-ASV table comprises 490 rows and 12065 columns, and the binary (presence/absence) data of this table were used to construct the network. We constructed 10 local networks representing plant-bacteria associations at site level, 3 regional networks for temperate, subtropical and tropical regions, and a global network using 'igraph' package in R. The regional and global networks were visualized using program GePhi with the 'ForceA-tlas2' layout algorithm, where plant species from different sites were highlighted using warm colors. For these networks, we identified hub ASVs in core positions of the networks by calculating the betweenness centrality score of each node/ASV. Betweenness centrality was standardized using the formula $(x - x_{min})/(x_{max} - x_{min})$ to make it range from 0 to 1, and ASVs with scaled betweenness above 0.5 were recognized as hubs[26]. A total of 115 ASVs were identified as metacommunity-level hubs at regional and/or global scales.

The biogeography of the hub ASVs was first explored using a heatmap that mapped the frequency of ASVs (the proportion of samples in which the ASVs occurred) for each site. We then applied a joint species distribution modeling approach, called Hierarchical Modelling of Species Composition (HMSC)[45], to predict the abundance of the 115 hub ASVs at the global scale. We selected a set of habitat variables that indicate important mechanisms potentially

structuring bacterial community assembly, including 3 soil variables (total nitrogen, total phosphorus and water content), 3 climate variables (temperature, precipitation and isothermality), and 3 plant leaf traits (nitrogen, phosphorus and specific leaf area). As plant trait data were not available for all samples, 1242 out of 1453 samples with available plant trait data were used in this analysis. The 9 variables were treated as fixed terms to evaluate the effect of habitat conditions on ASVs, and two distance matrices representing the spatial and host phylogenetic relationships between samples were used as random terms to control for the effects of spatially and phylogenetically clustered data.

We applied a hurdle model, i.e., one model for presence-absence and another one for abundance conditional on presence, in order to quantify the drivers of both presence-absence and abundance of hub ASVs. We applied probit regression in the presence-absence model, and linear regression for transformed count data in the abundance model. The count data were transformed by declaring zeros as missing data, log-transforming, and then scaling the data to zero mean and unit variance within each species[45]. We fitted the HMSC model with the R-package Hmsc assuming the default prior distributions. We sampled the posterior distribution with five Markov Chain Monte Carlo (MCMC) chains, each of which was run for 37,500 iterations, of which the first 12,500 were removed as burn-in. We examined MCMC convergence by examining the potential scale reduction factors of the model parameters. The MCMC convergence of our model was satisfactory: the potential scale reduction factors for the β-parameters (that measures the responses of the species to environmental covariates) were on average 1.013 and 1.04 for abundance and presence-absence model respectively. To compare the species distribution patterns of ASV among regions, we calculated the relative importance of each mechanism: the fixed effects of host traits, soil condition and climate, and the random effects of space and host

phylogeny, which were compared among temperate, subtropical and tropical hubs using paired samples Wilcoxon tests.

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Author contribution

Z.W., F.H. and S.W.K. conceived the project; Z.W. conducted field sampling and phyllosphere data collection; Y.J., M.Z., C.C., Y.C., S.F., G.J., M.J., J.L., Y.L., Y.L., K.M., X.M., X.Q., X.W., X.W., H.X., W.Y., L.Z., Y.Z. collected and collated the metadata and assisted with fieldwork; Z.W. analyzed data with supported from S.W.K.; Z.W. drifted the manuscript with input from S.W.K. and F.H.; All authors contributed to editing of the manuscript.

Competing interests

The authors declare no competing interests.

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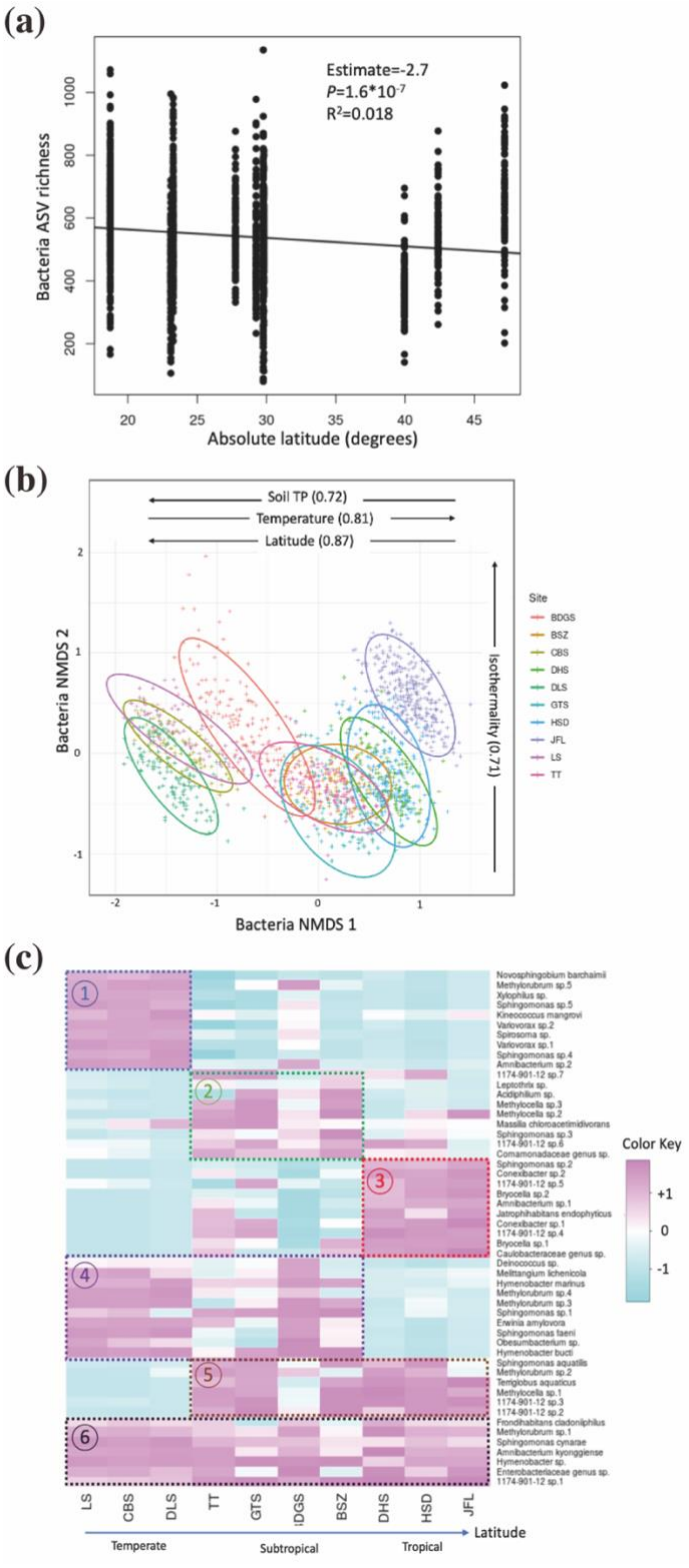
Figure 1 The latitude patterns of phyllosphere bacterial (a) diversity, (b) community composition and (c) occurrence of metacommunity-level hub ASVs. First- and second order polynomial fits are shown in black (dashed) and red (solid) respectively in (a), Bacterial community composition was visualized using nonmetric multidimensional scaling ordination with each ellipse representing site in (b), where arrows inside indicate significant variables correlated with ordination scores and the explained power (R^2) of the correlation. Heatmap in (c) show the relative frequency of hub ASVs clustering regarding to (1) temperate hubs (2) subtropical hubs (3) tropical hubs, (4) hubs that connect temperate and subtropical, (5) hubs that connect subtropical and tropical sites, and (6) global hubs that connect all sites.


(a)
Estimate=-2.7
P=1.6*10-7
R2=0.018
Bacteria ASV richness
Absolute latitude (degrees)
(b)
Soil TP (0.72)
Temperature (0.81)
Latitude (0.87)
Isothermality (0.71)
Site
BDGS
BSZ
CBS
DHS
DLS
GTS
HSD
JFL
LS
TT
Bacteria NMDS 2
Bacteria NMDS 1
(c)
Color Key
+1
0
-1
LS
CBS
DLS
TT
GTS
iDGS
BSZ
DHS
HSD
JFL
Latitude
Temperate
Subtropical
Tropical

544

Figure 2 Map of study sites indicating the percentage of variation in community composition explained by host, abiotic environment, neighborhood plants and spatial factors at local scales for each site, regional scales for temperate, subtropical, and tropical sites, and a 'global' scale that includes all sites. The Venn diagrams show the absolute variance explained by each factor and the histograms on the right of Venn diagrams show the relative contribution of the independent and shared effects of four variables to the explained variance. Explained variation less than 1% was not shown in the Veen diagram. Abbreviation; H: host, E: abiotic environments; S: spatial variables; N: neighborhood plant communities.

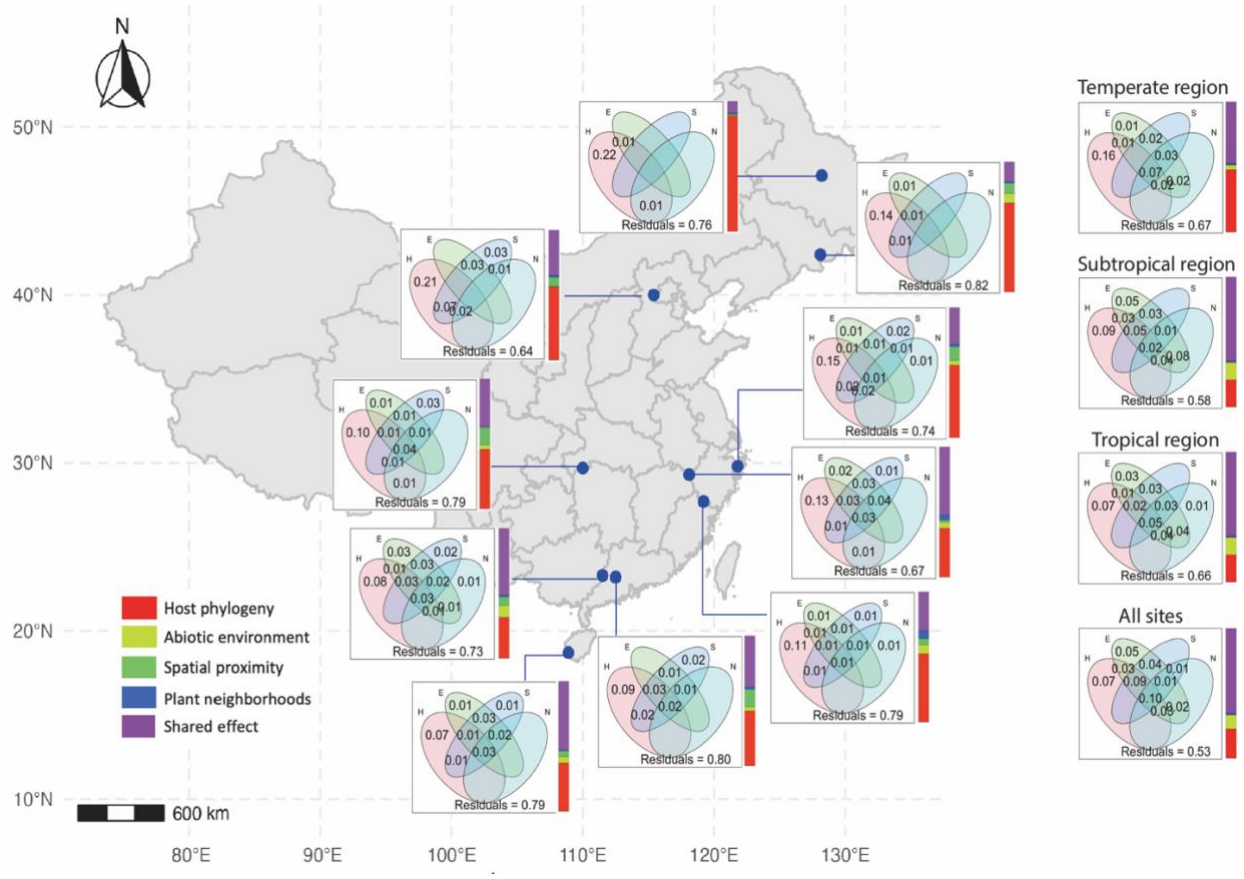


Figure 3 The relative importance of different mechanisms in structuring phyllosphere bacterial community assembly varies across latitude gradients (*a*-*d*) and across geographic scales (*e*). Coefficients of linear regression between the relative importance of drivers and latitude were reported in *a*-*d*, and Kruskal-Wallis rank sum test was applied to test the variation across three scales in *e*, showing moderate evidence (p=0.046) for increased confounding effects at larger scales (sample size: 10, 3 and 1 for local, regional and global scales).

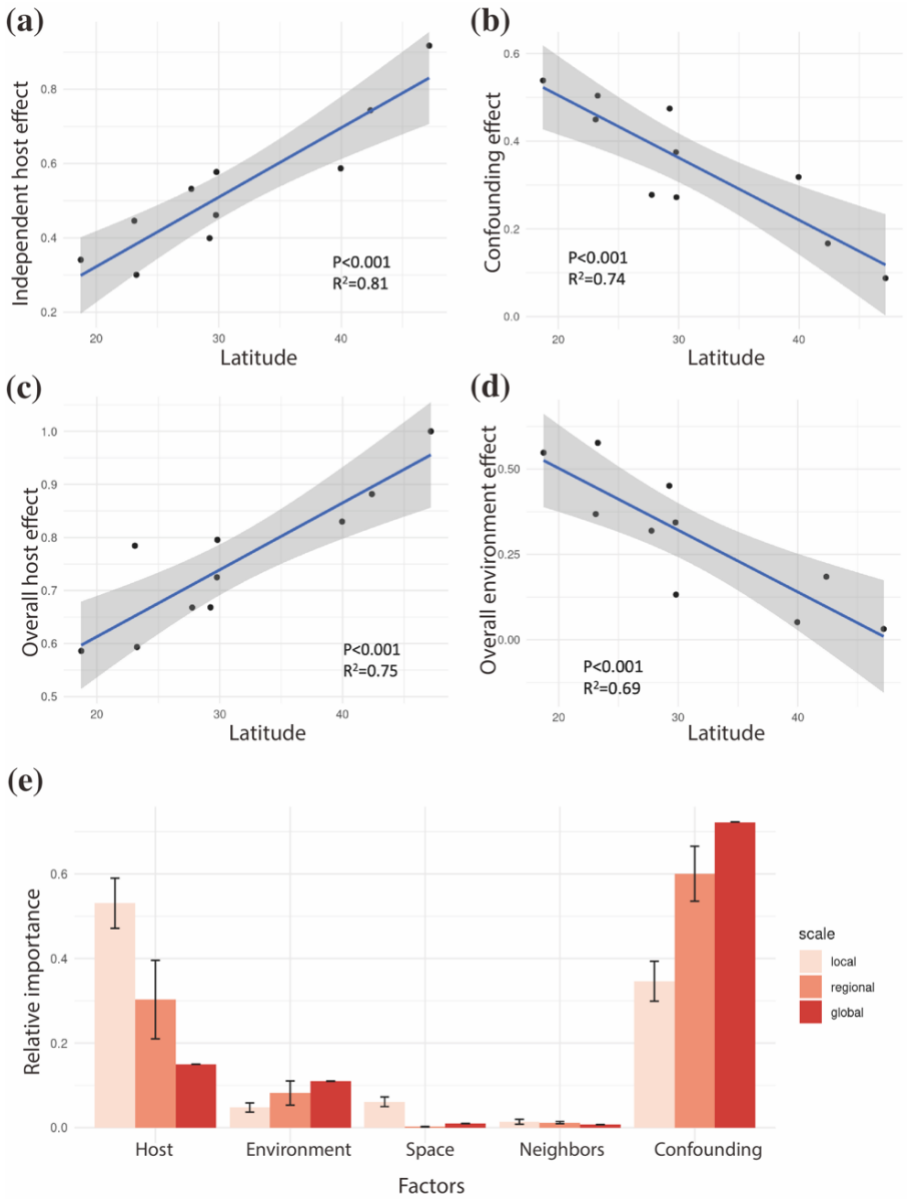


Figure 4 Global and regional networks representing the associations between plants and phyllosphere bacteria. Circles represent either bacteria ASVs (dark blue) or plant species (warm colors). A link was established if the plant species was associated with more than 15 reads of an ASV (see Methods). Size of the circles indicates the betweenness centrality of nodes. Plant species were colored according to latitude of site, including tropical: JFL, DHS, HSD; subtropical: BSZ, GTS, BDGS, TT; and temperate sites: DLS, CBS, LS.

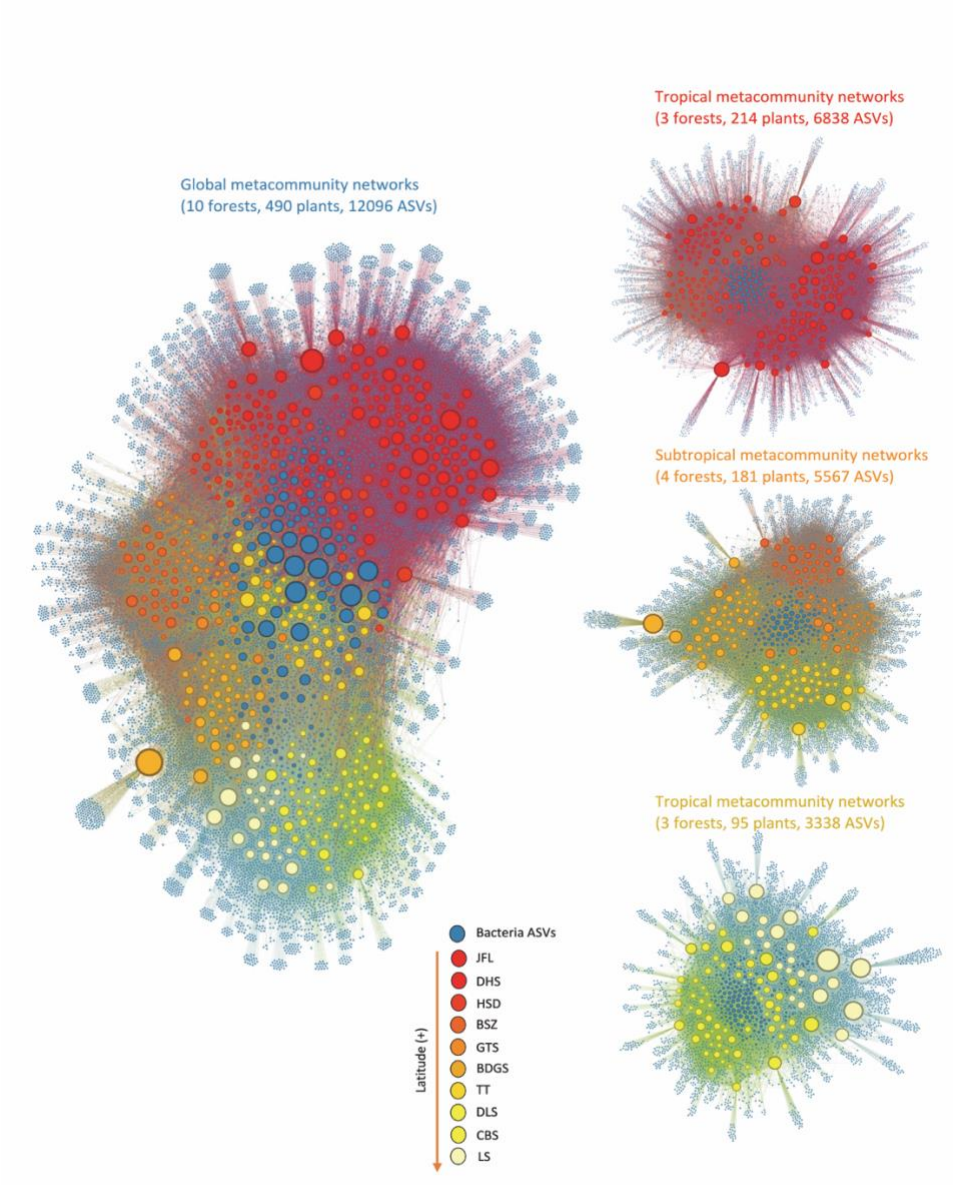


Figure 5 The results of joint species distribution modeling, showing (a) the absolute variation in bacterial abundance explained by each factor, the relative contribution of (b) host plant traits and (c) spatial factors on determining the abundance of ASVs in different regions. 115 ASVs were included in the model, and 9 habitat covariates and two distance matrices representing spatial and host phylogenetic relationship among samples were used as fixed and random term respectively. Each bar represents a hub bacterial ASV sorted according to the total variance explained, and the caption above the bar plot shows the averaged variation explained by each factor.

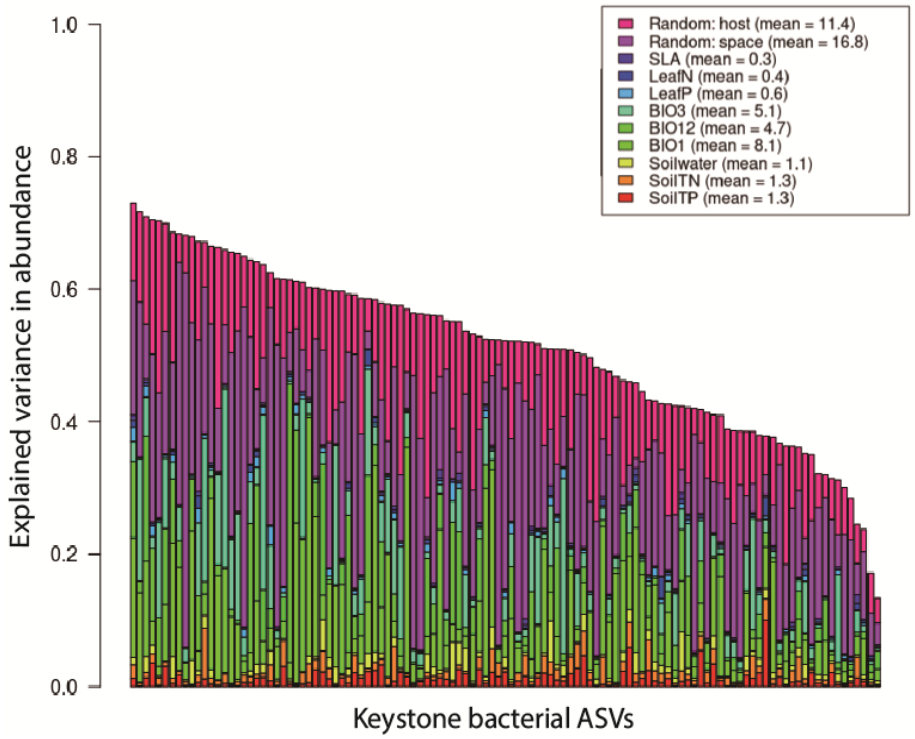
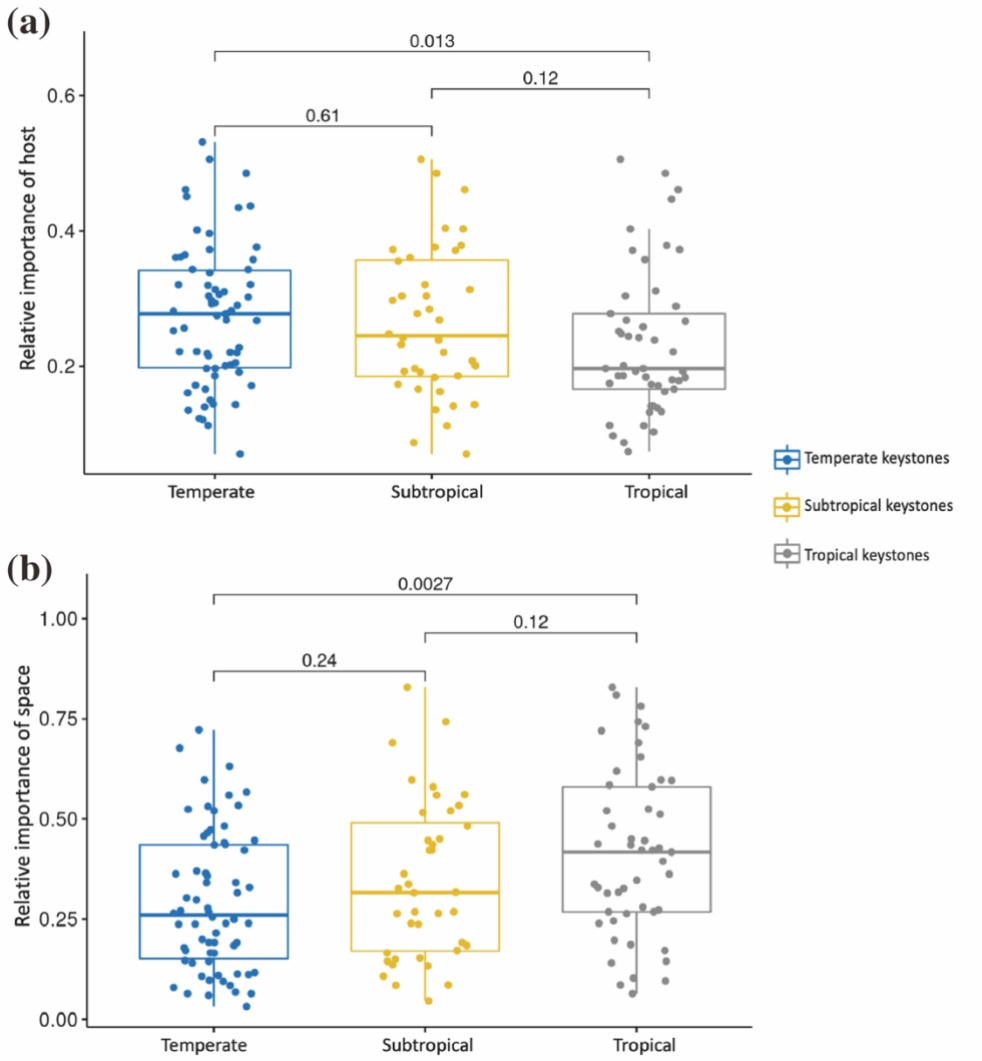



Figure 6 The contribution of host attributes (a) and spatial-related factors (b) to the abundance of hub taxa varies among regions. Numbers above the paired boxes symbolize the p values of pairwise Wilcoxin tests.