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Phytochemical diversity, endemism and their adaptations to abiotic and biotic pressures in fine roots across a climatic gradient

Running title: Rhizosphere phytochemicals and ecological adaptations

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Author Contribution

J. Y., Y.Z.Z. designed the study. Y.Z.Z, X.Y.S., M.C. and S.J.X. compiled and curated the data. Y.Z.Z., Y.Y.H., X.Z.W. and X.Z.W. analyzed the data. J.Y., Y.Z.Z. and S.J.W. wrote the first draft of the manuscript. All authors contributed to the several revisions of the manuscript.

Data Availability

Raw microbial sequences are deposited in the National Genomics Data Center under Bioproject fungi CRA006600 and bacteria CRA006619. Molecular network and associated MS data can be found at http://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=bfc7bf26d3b14c72ba48921f9f99b1f0. All methods and processes can be obtained directly from the online databases or publications described in the Methods. Data files and R code used in this study will be available in Figshare: https://doi.org/10.6084/m9.figshare.21804363.v1.

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Abstract

Phytochemicals are greatly ignored in trait-based ecology. Especially, the adaptations of phytochemicals to abiotic and biotic pressures in the rhizosphere are less understood. Here, we measured the metabolomics of fine roots and their rhizosphere microbiome along a climatic gradient (tropical, subtropical, and subalpine forests), to explore phytochemical diversity patterns and phytochemical-microorganism interactions. We found that high phytochemical diversity but low phytochemical endemism in subalpine species favor coping with high abiotic pressures. High phytochemical variation and phytochemical endemism in tropical species favor greater species coexistence and adaptation to complex biotic pressures. Moreover, there was evidence of widespread chemical niche partitioning of closely related species in all regions. Our findings support the Latitudinal Biotic Interaction Hypothesis, i.e., the intensity of phytochemical-microorganism interactions decreases from tropical to subalpine regions, which promotes greater multi-trophic coexistence in the tropics than in higher latitude forests. Our study provides novel insights into biotic interactions and species coexistence.

Introduction

Phytochemical diversity is referred to as the richness and abundance of specific metabolites produced by plant species or community (Defossez et al., 2021). It plays vital roles in determining plant adaptation and fitness (Rosenthal & Berenbaum, 1991) and ecosystem functions and services (Bruneton, 1995; Hunter, 2016). However, phytochemicals and their ecological adaptations are greatly ignored in trait-based ecology (Walker et al., 2022). Over the past decade, ecologists have explored the various aspects of phytochemical diversity and greatly improved our understanding of the ecological consequences of phytochemicals, e.g., variations in specific classes of metabolites across taxa (Cacho et al., 2015), their ecological significance for species coexistence and assembly (Kursar et al., 2009; Richards et al., 2015; Wang et al., 2022), and predictability of their spatial and evolutionary patterns (Defossez et al., 2021). However, previous studies have paid more attention to leaves, while phytochemicals and their adaptations to abiotic and biotic pressures in the rhizosphere are much less understood.

Fine (or absorptive) roots act as resource-acquiring organs and are essential for plant fitness and biogeochemical cycles (Laliberte, 2017). Fine roots, microorganisms, and soil further form the complex interface known as the rhizosphere (Dessaux et al., 2009). Rhizosphere phytochemicals link plants, soil, and microorganisms (Yin et al., 2018) and play an important role in defense against stressful abiotic conditions and biotic pressures (Rasmann et al., 2014; Oburger & Jones, 2018). However, there is still a lack of community-level studies on phytochemicals of fine roots in natural

ecosystems (e.g., forests) (Viant et al., 2017). Two opposite paradigms shed light on predicting phytochemical niche partitioning in fine roots. One hypothesis proposes that closely related plant hosts possess similar natural enemies, thus evolving similar chemistry (Berenbaum & Zangerl, 1998). An alternative hypothesis suggests that closely related species have divergent phytochemical defenses caused by natural selections of natural enemies (Webb et al., 2006), i.e., chemical niche partitioning. The latter has been widely supported by recent studies in leaves, i.e., the interactions of plants with their herbivores provide a high number of chemical niche dimensions and enhance plant diversity or coexistence (Kursar et al., 2009; Endara et al., 2021; Wang et al., 2022). However, whether fine roots also show similar chemical niche partitioning that promotes coexistence and how roots and microorganisms interact is a "black box".

Phytochemical diversity should be an ecological consequence of plant adaptation to abiotic and biotic conditions (Sedio et al., 2017). For example, cold and resource-poor environments can constrain plant growth, thus driving the selection of similar chemical defenses among species that reduce tissue loss (Coley et al., 1985). Natural enemies could drive divergent selection between related plants, leading to increasing chemical dissimilarity (Kursar et al., 2009). A study conducted along an elevational gradient indicated that low elevation regions with stable and productive habitats and higher biotic pressures favored increasing phytochemical diversity for protection, and high elevation regions with less biotic pressures had lower overall phytochemical diversity but increased phytochemical endemism (i.e., selection for

endemic molecules crucial for survival in stressful environments) (Defossez et al., 2021). However, the lack of large-scale studies (e.g., across latitude/climatic zones) limits our understanding of phytochemical diversity patterns and their adaptations or interactions with abiotic pressures or natural enemies, especially in the rhizosphere. Based on the Latitudinal Biotic Interaction Hypothesis (LBIH), which suggests that the intensity of biotic interactions increases from high to low latitudes (Schemske et al., 2009), we generally predict that the intensity of rhizosphere phytochemical-microorganism interactions should increase from cold and resource-poor regions to warm and resource-rich regions.

In this study, we measured the untargeted metabolomics of fine roots and the rhizosphere microbiome of 315 tree species along a macro-climatic gradient spanning tropical, subtropical, and subalpine forest ecosystems to explore the following questions. (i) What are the phytochemical diversity and endemism patterns of fine roots across a climatic gradient? (ii) Is there evidence of chemical niche partitioning of fine roots of related species and does it differ among climatic zones? (iii) What are the relationships between phytochemical diversity or endemism and rhizosphere microorganisms and how do they change across climates?

Materials and Methods

Study sites and sampling procedures

In 2012, we established a series of elevational gradient field experimental platforms that span tropical (21°36′ N, 101°34′ E), subtropical (24°32′ N, 101°01′ E),

and subalpine (27°08′ N, 100°12′ E) climatic gradients (**Fig. S1**, Song et al., 2021). The tropical platform represents montane rainforest ecosystems and includes four elevational transects: 800 m, 1000 m, 1200 m, and 1400 m. The subtropical platform represents mid-montane wet evergreen broad-leaved forest ecosystems and includes four elevational transects: 2000 m, 2200 m, 2400 m, and 2600 m. The subalpine platform represents coniferous forest ecosystems and includes four elevational transects: 3200 m, 3400 m, 3600 m, and 3800 m. The elevation span at each region generally covers the upper and lower limits of the distribution of forest ecosystems, which also represents the undisturbed natural forests. Each elevational transect has five 20 m × 20 m plots for a total of 60 plots studied. The latitudinal span of three climate zones is about six degrees (ca. 600 kilometers). Due to huge differences in their basic/initial elevations, a complete climatic gradient is formed within the local region, which is equivalent to these climatic changes across the large-scale latitudinal gradient (Song et al., 2021).

The plots were evaluated for vegetation features, climatic variables, and soil properties (details see **Supporting Information 1**). To match the specific environment data of each tree species in each elevational transect, we calculated the mean climatic and soil values of all plots where this tree species occurred.

Field sampling was conducted during the growing season from July to October in 2021. In each elevational transect, the root systems of every tree species were sampled. We selected 3-5 individuals from each species and collected fine roots of each individual from three random directions. Recently, the root-order definition has

been widely accepted as providing a more precise cutoff between absorptive and transport roots (McCormack et al., 2015; Freschet et al., 2021). For root phytochemicals, the first three orders are a reasonable and representative cutoff of fine (or absorptive) roots (Sun et al., 2021). Thus, we sampled fine roots of tree species, which were excavated by the root-tracing method (Laliberte, 2017). At the same time, the rhizosphere soil samples (soil attached to the fine roots) were carefully collected with a soft brush. The fine root and rhizosphere soil samples were homogenized to the tree species level at each elevational transect. In total, 315 tree species/samples were collected (**Supporting Information 1 Table S1**). Soil was sieved (2 mm) and stored in a portable freezer in the field and then transferred into a -40 °C freezer for microbial molecular analysis. Root samples were stored in liquid nitrogen in the field and then transferred into a −80 °C freezer in the laboratory for metabolome measurements.

Phytochemical metabolome measures

Secondary metabolites of plants are important phytochemicals for resisting abiotic pressures and natural enemies (Richards et al., 2015). Secondary metabolites in fine roots were extracted and analyzed following the procedure in Sedio et al. (2018a) with slight modification (also see Wang et al., 2022) (details see **Supporting Information 1**). The original UHPLC−MS/MS data were analyzed using the Global Natural Products Social (GNPS) Molecular Networking Metabolomics Tool (Wang et al., 2016). Based on the outputs of GNPS, we calculated two indexes, phytochemical

diversity and phytochemical endemism. Phytochemical diversity was represented by the Shannon – Wiener diversity index (i.e., alpha diversity), which was calculated using the abundance matrix of species samples (row) vs. compounds (column) using the "vegan" package (Oksanen et al., 2015). Phytochemical endemism reflects the dissimilarity of phytochemicals between different species (i.e., beta diversity), that is higher phytochemical endemism indicates lower similarity between local species, and thus possession of more endemic molecules (Defossez et al., 2021). We calculated phytochemical endemism by quantifying all pairwise combinations of compounds to calculate the chemical structural and compositional similarity (CSCS) scores for each pairwise combination of all 315 tree species following Sedio et al. (2017) and Wang et al. (2022).

Rhizosphere microbiome measures

Detailed processes of DNA extraction, amplification and PCR reactions are available in **Supporting information 1**. The library preparation and paired-end Illumina MiSeq sequencing was conducted using NovaSeq 6000 SP Reagent Kit by Personal Biotechnology Co., Ltd. (Shanghai, China). Bioinformatic analyses were performed using tools from the Quantitative Insights Into Microbial Ecology pipeline (QIIME2 version 2.0) (Bolyen et al., 2019). Raw sequence data were demultiplexed using the Demux plugin, and primers were cut off of reads with the Cutadapt plugin (Martin, 2011). Sequences were quality filtered, denoised and merged, and sequence chimeras were removed using the DADA2 plugin (separately for fungi and bacteria)

(Callahan et al., 2016). Non-singleton amplicon sequence variants (ASVs) were aligned with MAFFT (Katoh et al., 2002) and used to construct a phylogeny with FASTTREE2 (Price et al., 2010). All samples were resampled using the QIIME feature-table rarefy function, referring to the lowest sequence reads among all samples for downstream analyses. Plant pathogens were classified as fungal pathogens and bacterial pathogens. Fungal pathogens were extracted from ASVs based on the FUNGuild database (Nguyen et al., 2016). Those with a "confidence ranking" of "highly probable" were assigned as fungal pathogens. Bacterial pathogens were extracted from ASVs based on the Functional Annotation of Prokaryotic Taxa (Faprotax) database (Louca et al., 2016). We calculated the Shannon – Wiener diversity index of the rhizosphere sample of each tree species for total fungi, fungal pathogen, total bacteria, and bacterial pathogen using the "vegan" package (i.e., alpha diversity). Based on rhizosphere total fungi, fungal pathogen, total bacteria, and bacterial pathogen data, we calculated the paired Bray−Curtis dissimilarity matrix of tree species to represent the dissimilarity of microbial community composition (i.e., beta diversity) (Zhang et al., 2022a) using the "betapart" package (Baselga et al., 2021).

Statistical analyses

All statistical analyses were conducted in R 4.1.2 (R Core Team, 2021). The elevational patterns of phytochemical diversity were fitted using multinomial linear regressions in tropical, subtropical, and subalpine regions separately. The

comparisons of phytochemical diversity and endemism between different climatic regions were performed using the Wilcoxon test. Ordinal least square regressions were used to fit the relationships between phytochemical diversity and Shannon – Wiener diversity index of rhizosphere total fungi, fungal pathogen, total bacteria, and bacterial pathogen in different climatic regions and their total, respectively. We further fit the relationships between phytochemical diversity and the Shannon–Wiener diversity index of rhizosphere microorganisms using linear mixed effect models by the "nlme" package (Pinheiro et al., 2022). Principal component analysis (PCA) of climatic factors and soil properties was carried out to extract the first axis (PC1, explained variance > 70%) using the "FactoMineR" package (Sebastien et al., 2008). Phytochemical diversity was the response variable in the linear mixed effect models, climatic factors (PC1), soil properties (PC1), and microbial Shannon – Wiener diversity were predictor variables (fixed effects), and tree species ID and elevation were random effects. Then, we used hierarchical partitioning methods to quantify the relative importance of each predictor in the linear mixed effect models using the "glmm.hp" package (Lai et al., 2022).

We constructed the phylogenetic tree for plant species in each climatic region using Scenario 3 of V. PhyloMaker2 (Jin & Qian, 2022). It is the largest dated tree of life for vascular plants and is widely used in ecology (Zhang et al., 2022b). Phylogenetic distances between paired tree species were calculated using the "ape" package (Paradis & Schliep, 2018). Then, Mantel tests were used to test the relationships between phylogenetic distances and phytochemical similarities using the

235 "vegan" package. For our phytochemical traits (i.e., endemism/dissimilarity), the
236 number of trait dimensions always exceeds the number of species in a phylogeny
237 (Wang et al., 2022). Thus, K_{mult} was calculated to evaluate phylogenetic signals in
238 high-dimensional multivariate traits (Adams, 2014). Using random simulations based
239 on Brownian motion, K_{mult} values < 1 imply that the phenotypes of species resemble
240 each other less than expected under Brownian motion, whereas K_{mult} values > 1 imply
241 that the phenotypes of closely related species are more similar to one another than
242 expected under Brownian motion. Moreover, we also explored the relationships
243 between microbial community dissimilarities (Bray – Curtis) and phytochemical
244 endemism using Mantel tests.
245
246 **Results**
247 ***Phytochemical diversity and endemism patterns***
248 Phytochemical diversity and endemism patterns are shown in **Fig. 1**. In tropical
249 regions, phytochemical diversity showed an obvious hump pattern along elevation
250 (i.e., unimodal pattern at middle elevation), and 1200 m had the highest mean value of
251 phytochemical diversity (**Fig. 1a**). In subtropical regions, phytochemical diversity
252 also showed a hump pattern along elevation, and 2200 m had the highest mean value
253 of phytochemical diversity (**Fig. 1a**). In subalpine regions, phytochemical diversity
254 showed a slight hump pattern along elevation, and 3600 m had the highest mean value
255 of phytochemical diversity (**Fig. 1a**). Within each region, the elevational patterns of
256 phytochemical diversity were inconsistent with those of biodiversity (i.e., tree species,

fungi and bacteria showed U-shaped or monotonic decreasing patterns, **Fig. S2&3**). Subtropical regions had the highest values of phytochemical diversity (*P<0.001*), followed by subalpine regions, and tropical regions had the lowest values of phytochemical diversity (*P<0.001*) (**Fig. 1b**), with a hump pattern in phytochemical diversity across the climatic regions. However, tropical regions showed the highest variation/range in phytochemical diversity, followed by subtropical regions (**Fig. 1a&b**).

Subtropical regions had the lowest phytochemical similarity, followed by tropical regions, and subalpine regions had the highest phytochemical similarity (**Fig. 1c**). Phylogenetic distance and phytochemical similarity showed significant, positive correlations in all climatic regions, i.e., subtropical (r=0.63, *P<0.001*), subalpine (r=0.61, *P<0.001*) and tropical (r=0.51, *P<0.001*) (**Fig. S4**), which means that more closely related plant species showed higher phytochemical dissimilarity/endemism. Moreover, phytochemicals also showed no phylogenetic signals in all climatic regions: subalpine (K_{mult} = 0.064, *P>0.05*), subtropical (K_{mult} = 0.025, *P>0.05*) and tropical (K_{mult} = 0.121, *P>0.05*). These results suggest that phytochemicals of fine roots are not phylogenetically conserved in any of the climatic regions.

The relationships between phytochemical diversity and microbial diversity

At the species level, total fungi diversity showed no significant relationship with phytochemical diversity in all cases (*P>0.05*) (**Fig. 2a**). Fungal pathogen diversity and phytochemical diversity showed no significant relationship in subalpine regions (R^2=0.016, *P>0.05*), a significant, negative relationship in subtropical regions

(R^2=0.19, *P<0.05*), no significant relationship in tropical regions (R^2=0.11, *P>0.05*), but a significant, negative relationship in total climatic regions (R^2=0.21, *P<0.001*) (**Fig. 2b**). Total bacteria diversity and phytochemical diversity showed no significant relationship in subalpine regions (R^2=0.12, *P>0.05*), but significant, negative relationships in subtropical (R^2=0.2, *P<0.05*), tropical (R^2=0.28, *P<0.001*), and total climatic regions (R^2=0.37, *P<0.001*) (**Fig. 2c**). Bacterial pathogen diversity showed no significant relationship with phytochemical diversity in all cases (*P>0.05*) (**Fig. 2d**).

In the mixed effect models with phytochemical diversity as the response variable and climatic factors, soil properties, and microbial diversity as predictor variables, we found that the importance of microbial diversity in total explained variance generally decreased from tropical regions to subalpine regions and microbial diversity was only significant in the tropics (*P<0.05*) (**Fig. 3**), which also supports our results that reflect changing phytochemical-microorganism interactions across latitude **(Fig. 2)**. Climatic factors showed significant effects on phytochemical diversity in all regions and soil properties showed significant effects on phytochemical diversity only in the subtropical region. However, the importance of abiotic factors in total explained variance increased from tropical regions to subalpine regions (**Fig. 3**), which reflects changing abiotic responses. In particular, climatic factors were the most important predictor of phytochemical diversity in subalpine regions, which reflects the huge effects of temperature and water stress on phytochemicals.

The relationships between phytochemical endemism and microbial dissimilarity

At the species level, total fungi community dissimilarity and phytochemical endemism showed no significant relationship in subalpine regions (r=0.04, *P>0.05*), a significant relationship in subtropical regions (r=0.17, *P<0.001*), and a significant relationship in tropical regions (r=0.28, *P<0.001*) (**Fig. 4a**). Fungal pathogen community dissimilarity and phytochemical endemism showed no significant relationship in subalpine regions (r=0.004, *P>0.05*) or subtropical regions (r=0.04, *P>0.05*), but a significant relationship in tropical regions (r=0.25, *P<0.001*) (**Fig. 4b**). Total bacteria community dissimilarity and phytochemical endemism showed significant relationships in all regions, subalpine (r=0.12, *P<0.05*), subtropical (r=0.24, *P<0.001*), and tropical (r=0.26, *P<0.001*) (**Fig. 4c**). Bacterial pathogen community dissimilarity and phytochemical endemism showed no significant relationship in subalpine regions (r=0.08, *P>0.05*), no significant relationship in subtropical regions (r=0.04, *P>0.05*), and a significant relationship in tropical regions (r=0.25, *P<0.001*) (**Fig. 4d**).

Discussion

Spatial patterns of fine root phytochemicals and their ecological significance

Understanding how phytochemicals vary across ecosystems is key to uncovering ecological indicators and functions of phytochemicals in landscapes (Bruneton, 1995; Sedio et al., 2017). However, so far, most studies have focused on specific-taxa (e.g.,

322 *Inga* and *Piper*) (Kursar et al., 2009; Richards et al., 2015) in a single community/plot
323 (e.g., Euphorbiaceae in a plot) (Wang et al., 2022) or in a region (e.g., *Inga* across the
324 Amazon) (Endara et al., 2021). Studies involving analyses of phytochemicals across
325 many communities or across climatic regions are uncommon due to difficult sampling
326 and limited methods. A recent regional study focusing on grassland communities
327 along an elevational gradient revealed the spatial predictability of foliar
328 phytochemical diversity (Defossez et al., 2021). They found that low elevation
329 habitats with less stressful abiotic environments, but greater biotic pressures favor
330 increased phytochemical diversity for protection (Coley et al., 1991), while high
331 elevation regions with heterogeneous habitats favor decreased phytochemical
332 diversity but select specific molecules essential for survival in stressful environments
333 (i.e., higher phytochemical endemism) (Jacobo-Velázquez & Cisneros-Zevallos,
334 2018). However, our study across large-scale climatic gradients and focused on fine
335 roots revealed a different pattern (**Fig. 1b**), i.e., subalpine regions showed higher
336 phytochemical diversity than tropical regions. This result, i.e., higher chemical
337 defenses at higher latitudes or elevations, is supported by previous studies (Moles et
338 al., 2011), and noted as a positive adaptation to stressful environments (high
339 ultraviolet, high wind, and freezing cold) (Rasmann et al., 2014). It is worth noting
340 that tropical regions have higher variation in phytochemical diversity and greater
341 phytochemical endemism, whereas subalpine regions have less variation (i.e.,
342 consistently high diversity) and lower phytochemical endemism. Subalpine habitats
343 are less heterogeneous than the tropics but consistently/evenly harsh (Zhang et al.,

2021), thus these consistently harsh environmental pressures may lead to low variation in phytochemical diversity and low phytochemical endemism (selection for similar molecules crucial for survival in stressful environments). Conversely, tropical habitats are extremely heterogeneous in both abiotic and biotic environments (Polato et al., 2018), which is considered an important driver for high species coexistence (Janzen, 1967; Smith, 2018). Thus, we inferred that higher variation in phytochemical diversity and greater phytochemical endemism reflect the various chemical niches of fine roots used to adapt to heterogeneous habitats in the tropics.

Higher phytochemical dissimilarity/endemism in the tropics than in the subalpine regions (**Fig. 1c**) also supported the idea of chemical niche partitioning of tropical species. It provides rational evidence to support a widely accepted proposition: species coexisting in a local region are likely to differ in key ecological niche dimensions (Webb et al., 2006). The phytochemical niche dimension is a crucial but long-ignored aspect of species coexistence, which has been strongly supported by another recent study (Wang et al., 2022). The divergent phytochemical diversity and endemism patterns in different climatic regions may explain the species coexistence mechanism from a phytochemical perspective, e.g., tropical species possess greater chemical niche partitioning which promotes higher species coexistence. Our other results also support the idea of chemical niche partitioning between coexisting species in all climatic regions, i.e., more closely related species are more dissimilar in their phytochemicals, thus phytochemicals were not regulated by phylogeny. However, our findings contrast with Sedio et al. (2018b), who found that foliar phytochemicals were

366 phylogenetically conserved for temperate species but not for tropical species.
367 Importantly, our findings extend the universality of the chemical niche partitioning
368 hypothesis in regional species pools beyond specific taxa (Kursar et al., 2009), and
369 contrast with the prevailing coevolutionary theory: closely related plant species
370 should be similar in defense strategies (Ehrlich & Raven, 1994). Moreover, species
371 phylogeny alone poorly predicts phytochemical diversity, thus abiotic and biotic
372 pressures should be considered in shaping phytochemicals (Defossez et al., 2021).

373 We found an interesting phenomenon in mid-latitude regions, i.e., subtropical
374 regions had the highest phytochemical diversity and endemism (**Fig. 1b&c**). We
375 assume that plants in subtropical regions need to cope with mixed pressure from both
376 abiotic and biotic conditions, but tropical or subalpine species only cope with high
377 biotic pressure and negligible abiotic pressures or stressful environments and
378 negligible biotic pressures, respectively. Thus, subtropical species should evolve to
379 harbor two categories of phytochemicals to adapt to both abiotic and biotic pressures,
380 higher phytochemical diversity and endemism. This explanation also fits with the
381 hump pattern of phytochemical diversity along elevations in tropical and subtropical
382 regions (**Fig. 1a**), here we term this pattern the "middle enrichment phenomenon".

383 ***Intensity of phytochemical-microorganism interactions along climatic***
384 ***gradients***

385 The ubiquitous decrease in biodiversity from low to high latitudes is seen as one
386 of the fundamental ecological laws (Lawton, 1999). A latitudinal gradient in the
387 intensity of biotic interactions, to some degree, may explain the latitudinal pattern of

biodiversity (Zvereva & Kozlov, 2021), which proposed that the intensity of biotic interactions increases from high to low latitudes (Schemske et al., 2009), i.e., the Latitudinal Biotic Interaction Hypothesis (LBIH). In this scenario, current theory also predicts that plants from low latitudes will be better defended against natural enemies than those from high latitudes (MacArthur, 1972). However, most studies have focused on leaf herbivores, i.e., the intense biotic interactions at low latitudes will select plants with greater defenses against herbivores (Pennings et al., 2007; Moles et al., 2011), while few studies have involved rhizosphere natural enemies. Unfortunately, leaf studies often find conflicting latitudinal patterns of defense values or herbivory rates (Moles et al., 2011). Examples include higher herbivory at lower latitudes (Pennings et al., 2007), no relationship between herbivory and latitude (Adams et al., 2010), and higher herbivory at high latitudes (Gaston et al., 2004). Thus, it is infeasible to explore latitudinal trends in natural enemies or plant defenses alone to reveal the strength of biotic interactions.

For example, our findings suggest that factors affecting chemical defenses are the combined effects of abiotic and biotic pressures potentially leading to the highest phytochemical diversity in middle latitude regions. Therefore, it is suitable to evaluate the explanatory power or goodness of fit models for phytochemical diversity of microorganism/pathogen diversity to represent phytochemical-microorganism interactions, as applied in Nishizawa et al. (2022). Our results found that the explanatory powers of phytochemical-microorganism models decrease from tropical to subalpine regions (especially in fungal pathogens and total bacteria), which

supports the prediction of LBIH (**Fig. 2&3**). In contrast, although abiotic factors (climate or soil) showed significant effects on phytochemical diversity in all regions (Defossez et al., 2021), the importance of abiotic factors increased from tropical to subalpine regions (**Fig. 3**), which emphasizes the more positive adaptation of root phytochemicals to abiotic pressures in high latitudes. In addition to alpha diversity findings involved in the relationships between phytochemical and microbial diversity, we also provide similar evidence from beta diversity, i.e., phytochemical endemism vs. microbial dissimilarity. It is well established that herbivores and pathogens can promote divergent selection and increase chemical dissimilarity/endemism between closely related plants (Kursar et al., 2009). Our findings revealed the apparent latitudinal patterns of correlations between phytochemical endemism and microbial dissimilarity, i.e., the correlations decrease from tropical to subalpine regions. The latitudinal patterns of beta-level interactions also fit the prediction of LBIH, reflecting the changing intensity of biotic interactions along climatic gradients. It also supports the ecological significance of phytochemicals in fine roots for multi-trophic species coexistence (i.e., host plants and rhizosphere microorganisms), especially in low latitudes, that is, greater biotic interactions can further promote phytochemical niche partitioning of host plants and greater variation in rhizosphere microbial community composition (Zhang et al., 2022a).

Perspective of rhizosphere phytochemicals in community ecology

The rhizosphere is a complex system, that is faced with both abiotic pressures (e.g., temperature, water, and soil nutrient conditions) and biotic pressures (e.g.,

herbivores and microorganisms) (Yin et al., 2018) (**Fig. 5**). Phytochemicals of fine roots, as regulators of these pressures, can protect plant tissue against stressful abiotic conditions (Rasmann et al., 2014) and interact (e.g., defense) with soil microorganisms and recruit rhizosphere microbiomes (Sasse et al., 2018) (**Fig. 5**). Therefore, patterns of phytochemicals and their ecological significance are important clues to understanding biodiversity maintenance mechanisms and biotic interactions. Our study is a positive exploration into phytochemical diversity patterns and their interactions with rhizosphere microorganisms along climatic gradients, which highlights the importance of future phytochemical research. We propose some key points here that deserve attention in future rhizosphere ecology research.

(1) *Scale-dependence*. Many studies have found that the rhizosphere environment allows only certain microbial taxa to colonize or persist by secreting bioactive phytochemical molecules (Hu et al., 2018; Sasse et al., 2018). As such, it is widely held that plant-host dependency is the overwhelming control in rhizosphere microbial community assembly not only at micro-spatial scales but also at community scales. However, recent studies suggest that the diversity patterns and community assembly of host plants and rhizosphere microorganisms are often decoupled at community/regional scales. Limited available evidence also suggests that microbial community plant-host dependency at broad elevational scales may be weak (Fierer et al., 2011). Mycorrhizal fungi and their host plants show divergent β-diversity relationships along a latitudinal gradient (Zheng et al., 2021). Here, we provide additional evidence based on rhizosphere phytochemicals and their relationships with

rhizosphere microorganisms. For example, our significant results were found only based on species-level data (micro-spatial scales), and elevational patterns of plant, fungi and bacteria diversity are inconsistent with phytochemical diversity at community scales (**Fig. S2&3**). Therefore, it is necessary to select the research scale accurately in studying the ecological significance of phytochemicals. It also directs the design of future experiments, e.g., climatic and soil factors should be measured at the species level (but plot-level data were used in this study due to difficult collections) to explore the response of phytochemicals to abiotic pressures in future studies.

(2) *Various functional groups of microorganisms*. We found that total fungi diversity showed no clear relationship with phytochemical diversity, but fungal pathogen diversity did (negative). This indicates that other functional groups of fungi (e.g., ectomycorrhiza and arbuscular mycorrhiza) may show converse relationships (e.g., positive mutualism) with phytochemical diversity. However, Xia et al. (2021) suggest that fine root compounds show negative trade-off relationships with mycorrhizal colonization. These contrasting conclusions call for further exploration. Moreover, we found that total bacteria diversity showed clear negative relationships with phytochemical diversity. We inferred that some favorable bacteria (e.g., nitrogen-fixing and nitrifier) are also limited by the anti-bacterial properties of root chemistry. Moreover, ecologists should pay more attention to plant bacterial pathogens, as bacterial pathogens may have shown no significant relationship with phytochemical diversity in this study because too few plant pathogens were able to be annotated using Faprotax.

(3) *Specific classes of metabolites*. Aanthocyanin and numerous flavonoids will corroborate the protective role of these compounds against stressful abiotic conditions, e.g., high ultraviolet radiation and freezing cold (Rasmann et al., 2014). Phenolics and saponins were verified to have anti-herbivore effects (Kursar et al., 2009). Lignin, bound phenols, condensed tannins, hydrolysable tannins, flavonoids and total soluble phenolics were treated as anti-fungal protective compounds (Xia et al., 2021). However, so far, we still lack understanding of the specific relationships between different microbial taxa or functional groups and different classes of metabolites. Some studies have applied the divisions of specific classes of metabolites based on metabolomics in community ecology (Sedio et al., 2021), which may have the potential to push understanding of the ecological significance of phytochemicals.

(4) *Rhizosphere herbivores*. Herbivores are the most commonly focused on topic in leaf defenses (Wang et al., 2022), but are remarkably ignored in the rhizosphere. Below-ground ecological processes greatly lag behind that of above-ground processes because of the huge difficulty of implementation, especially in the collection and identification of soil herbivores (Gan & Wickings, 2020). We hope for more studies on rhizosphere phytochemical-herbivore interactions in the future.

Conclusions

It is well known that, along latitudes, climate (warm vs. cold) and resource (rich vs. poor) pressures both increase from tropical to subalpine regions, while biotic pressures decrease from tropical to subalpine regions (**Fig. 5**). These latitudinal/climatic gradients provide a macroecological background that we used to

explore the ecological causes and consequences of phytochemicals of fine roots. We found higher variation in phytochemical diversity and lower phytochemical similarity in tropical tree species while subalpine tree species harbored higher phytochemical diversity but lower phytochemical variation and dissimilarity, which plays important roles in coping with high biotic and abiotic pressures in tropical and subalpine regions, respectively (**Fig. 5**). Moreover, we found that mid-latitude regions (subtropical) have the highest phytochemical diversity and endemism potentially due to the combined effects of middle-level biotic and abiotic pressures compared with tropical and subalpine regions (**Fig. 5**). We also found widespread chemical niche partitioning of closely related species and that phytochemicals are not regulated by phylogeny but instead potentially by abiotic and biotic pressures. Lastly, our findings support the Latitudinal Biotic Interaction Hypothesis (LBIH), that is the intensity of phytochemical-microorganism interactions monotonically decreases from tropical to subalpine regions (**Fig. 5**). The intense phytochemical-microorganism interactions in the tropics promote greater variation in microbial community composition (greater beta diversity or species turnover, Zhang et al., 2022a) and phytochemical niche partitioning of host plants (higher phytochemical endemism) than that of higher latitudes, which shapes the enormous multi-trophic coexistence in the tropics. Our study reveals phytochemical diversity patterns and their ecological significance in fine roots and provides novel insights into biotic interactions and species coexistence.

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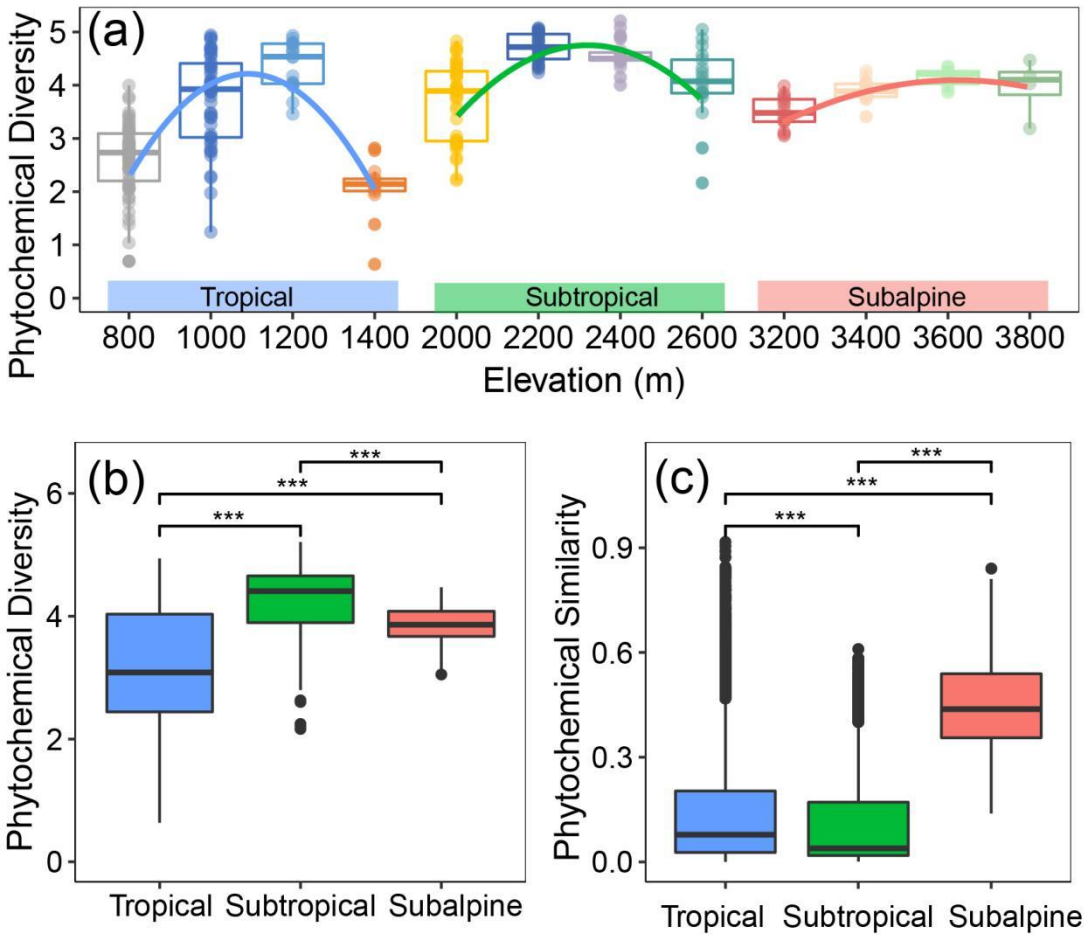


735

736 **Figure 1.** Phytochemical diversity and endemism patterns across climatic gradients.

737 (a) Phytochemical diversity patterns along elevation in different climatic regions.

738 Lines were fitted by multinomial linear regressions. (b) Phytochemical diversity

739 difference between different climatic regions. (c) Phytochemical endemism difference

740 between different climatic regions, i.e., low similarity means high endemism.

741 ****p*<0.001.

742

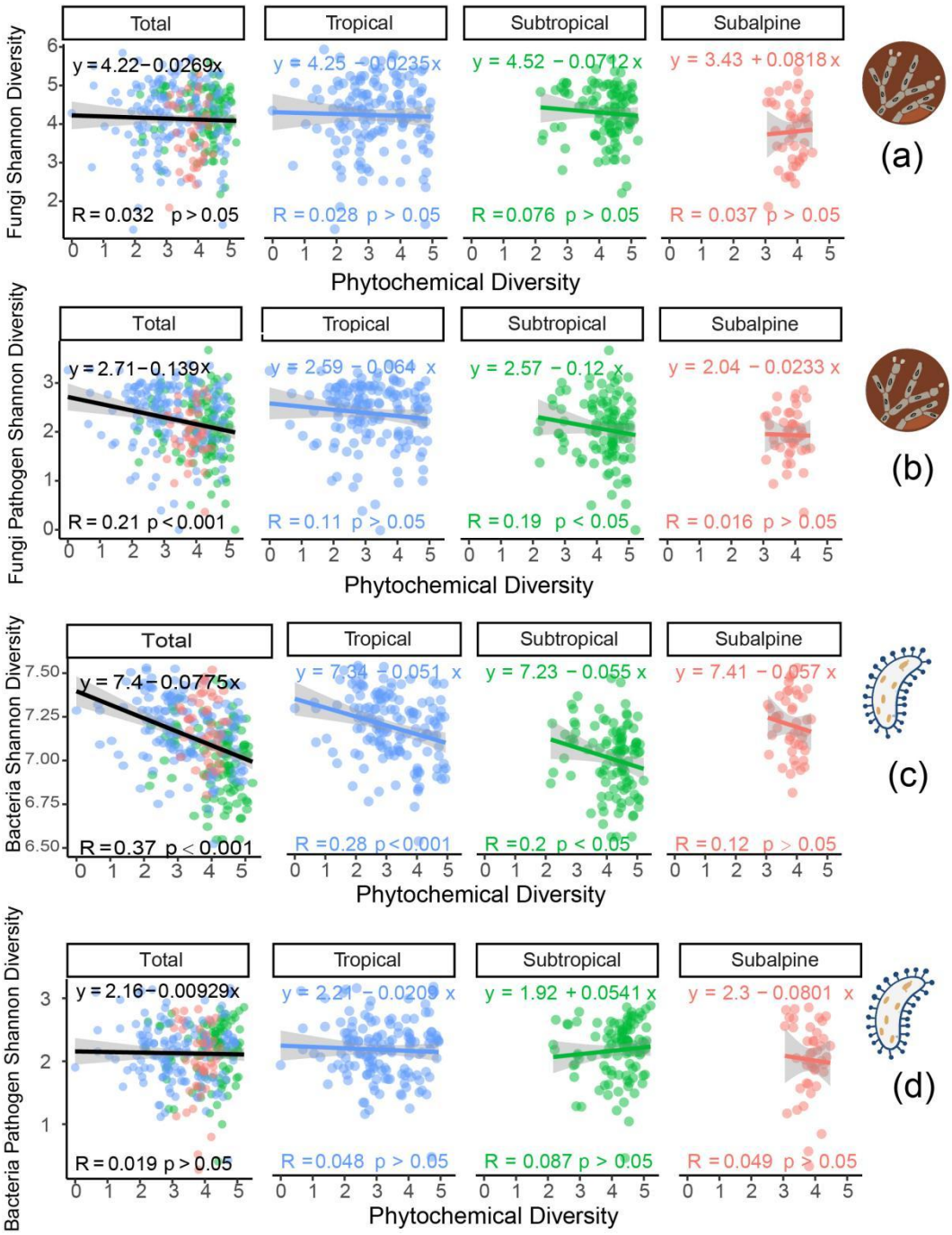


Figure 2. The relationships between phytochemical diversity and microbial diversity in total climatic regions, tropical, subtropical, and subalpine regions, respectively. (a) Total fungi diversity. (b) Fungal pathogen diversity. (c) Total bacteria diversity. (d) Bacterial pathogen diversity. Lines were fitted by ordinal least square regressions, and intercept, slope, R^2 and p value are given.

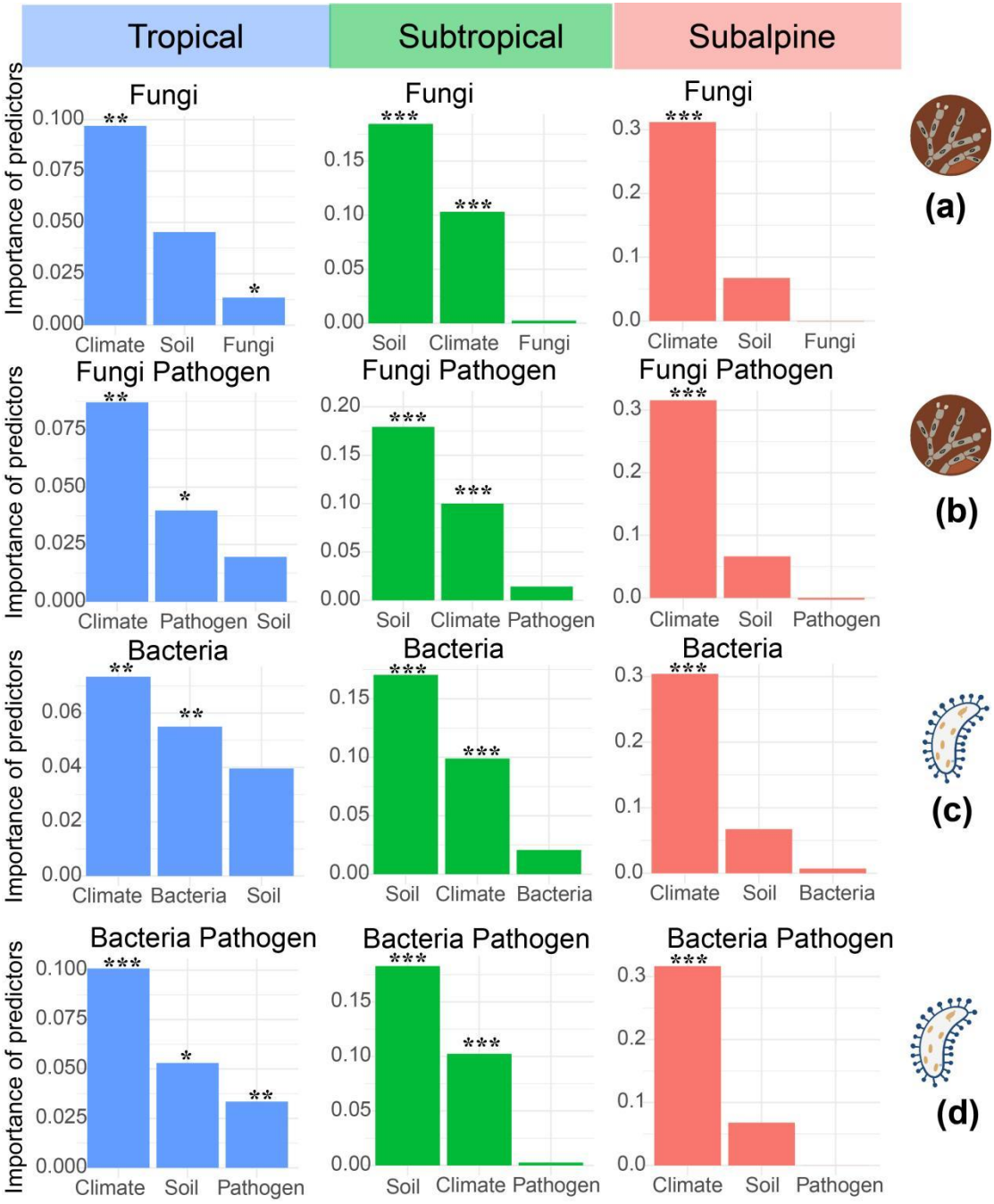
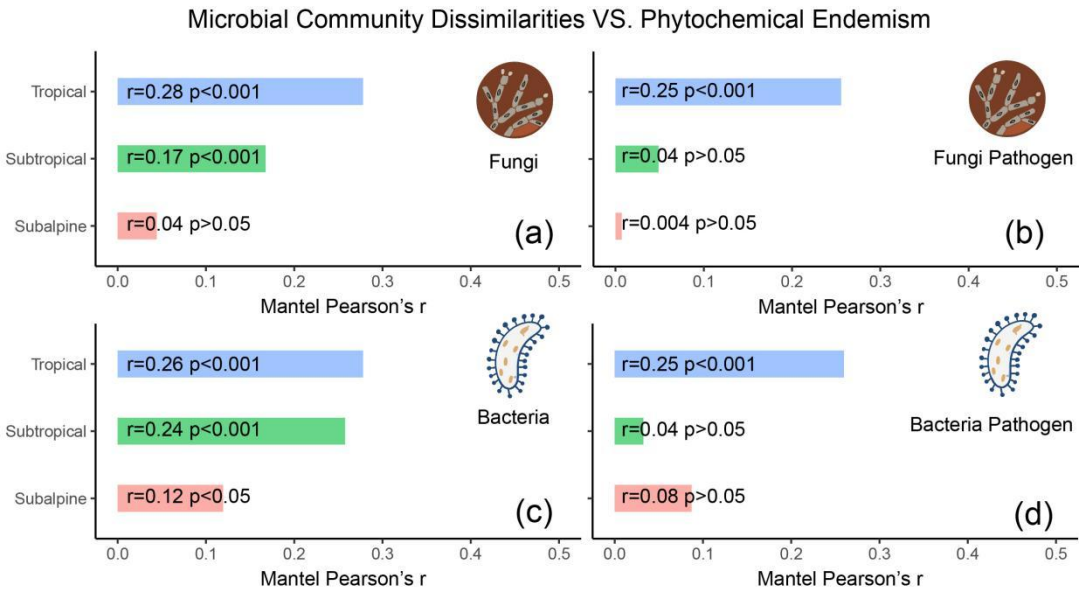


Figure 3. The relative importance of predictors that shape phytochemical diversity in different climatic regions based on hierarchical partitioning methods. (a) Total fungi diversity. (b) Fungal pathogen diversity. (c) Total bacteria diversity. (d) Bacterial pathogen diversity. Phytochemical diversity was the response variable, and climate, soil, and microbial diversity were predictor variables. Climate: the PC1 axis of temperature and humidity. Soil: the PC1 axis of soil properties.

757 fungi/bacteria/pathogen: the Shannon−Wiener diversity index of fungi, bacteria, and
758 pathogen. P values were resulted from mixed effect models, *$p<0.05$, **$p<0.01$,
759 ***$p<0.001$.
760



761

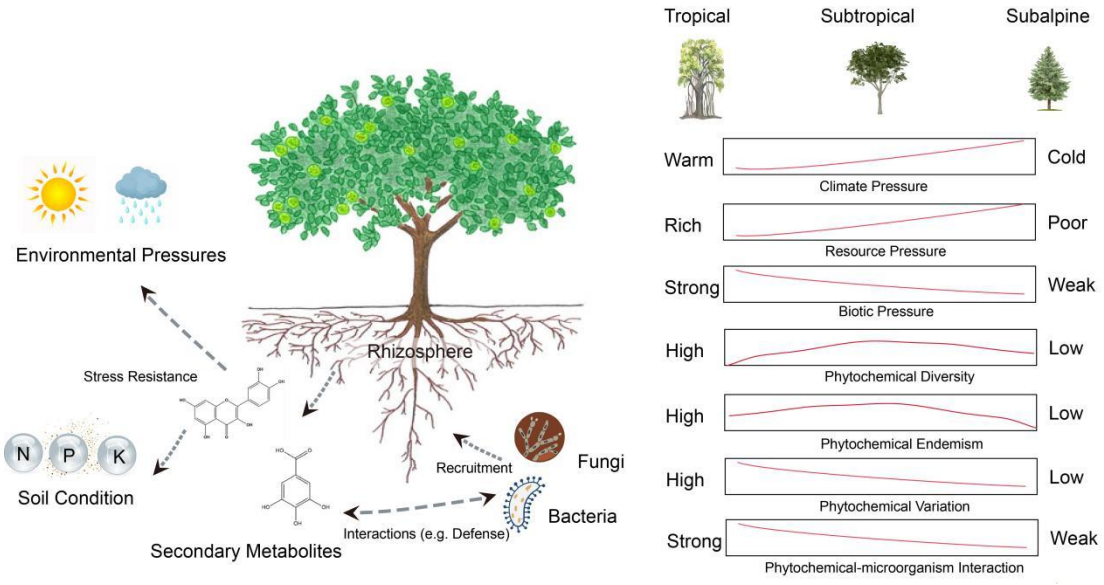
762 **Figure 4.** The relationships between microbial community dissimilarities and

763 phytochemical endemism in different climatic regions. (a) Total fungi dissimilarities.

764 (b) Fungal pathogen dissimilarities. (c) Total bacteria dissimilarities. (d) Bacterial

765 pathogen dissimilarities. Mantel Pearson's r and p value are given.

766



767

768 **Figure 5.** Diagram of phytochemical patterns and their relationships with abiotic and

769 biotic factors along climatic gradients. Climate and resource pressures reflect the

770 stressful conditions from environments. Biotic pressure reflects the effects from

771 natural enemies (e.g., herbivores and pathogens). Phytochemical variation reflects the

772 total phytochemical difference between local species. Phytochemical endemism

773 reflects the phytochemical dissimilarity between local species.

774