Science of the Total Environment 927 (2024) 172051

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

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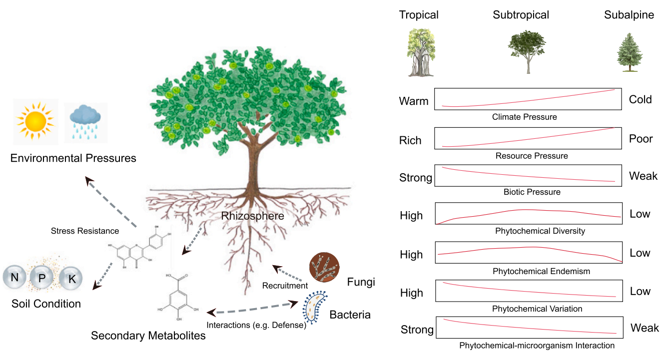
HIGHLIGHTS

- Higher phytochemical diversity but less endemism in subalpine species facilitate adaptation to challenging environments
- Higher phytochemical endemism in tropical species increases species coexistence and adaptations to various niches
- Closely related plant species in all climatic regions exhibit chemical niche partitioning
- Phytochemical-microorganism interactions decrease from tropical to subalpine regions

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Wei Shi

Keywords:
Metabolic pathway
Species coexistence
Biotic interaction
Rhizosphere microorganism
Metabolome

ABSTRACT

Phytochemicals and their ecological significance are long ignored in trait-based ecology. Moreover, the adaptations of phytochemicals produced by fine roots to abiotic and biotic pressures are less understood. Here, we explored the fine roots metabolomes of 315 tree species and their rhizosphere microbiome in southwestern China spanning tropical, subtropical, and subalpine forest ecosystems, to explore phytochemical diversity and endemism patterns of various metabolic pathways and phytochemical-microorganism interactions. We found that subalpine species showed higher phytochemical diversity but lower interspecific variation than tropical species, which favors coping with high abiotic pressures. Tropical species harbored higher interspecific phytochemical variation and phytochemical endemism, which favors 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, and phytochemicals showed a weak phylogenetic signal, but were regulated by abiotic and biotic pressures. 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 microbial community turnover and phytochemical niche partitioning of host plants in the tropics than in higher

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https://doi.org/10.1016/j.scitotenv.2024.172051
Received 17 August 2023; Received in revised form 16 March 2024; Accepted 26 March 2024

latitude forests. Our study reveals the convergent phytochemical diversity patterns of various pathways and their interactions with microorganism, thus promoting species coexistence.

1. Introduction

Phytochemical diversity is referred to as the complexity and the variation of phytochemical compositions produced by plant species or community (Wetzel and Whitehead, 2020). It plays vital roles in determining plant adaptation and fitness (Rosenthal and Berenbaum, 1991) and ecosystem functions and services (Bruneton, 1995; Hunter, 2016). However, phytochemicals and their ecological adaptations are long ignored in trait-based ecology (Walker et al., 2022). Compared to easily measured functional proxies, phytochemicals have a more direct, clearer functional significance in adaptations or interactions to abiotic and biotic environments (Xia et al., 2021). For example, alkaloids and terpenoids have important defense functions against microorganisms and herbivores (Matsuura and Fett-Neto, 2015; Toffolatti et al., 2021) and polyketides can defend ecological pressures (Coley et al., 1985). 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 and Jones, 2018). The prominent lags in rhizosphere phytochemicals inhibit the understanding of the mechanistic basis for the root trait adaptations, below-ground biotic interactions and biodiversity maintenance (Xia et al., 2021).

Phytochemicals of fine roots should be an ecological consequence of plant adaptations to abiotic and biotic conditions (Sedio et al., 2017). For example, tannins and flavonoids of roots can regulate below-ground biotic interactions, e.g., chemical protection against natural enemies and affecting microbial symbiosis (Solaiman and Senoo, 2018; Suseela et al., 2020). Some organic acids, e.g., jasmonic acid and salicylic acid, have shown to mediate abiotic stress (e.g., salinity, drought and thermal stress) responses in roots (Sanchez-Bermudez et al., 2022). However, the variation of fine root chemistry along large-scale environmental gradients is still poorly understood. Latitudinal and elevational gradients both provide ideal backgrounds for studying phytochemical patterns of plants growing under contrasting biotic and abiotic conditions (Sedio et al., 2018a; Volf et al., 2022). However, phytochemical diversity patterns along the above gradients predicted by classical theories may be inconsistent with some empirical observations. First, some theories indicated that cold and resource-poor environments can constrain plant growth, thus driving the selection of increasing chemical defenses (Moles et al., 2011), which should lead to higher phytochemical diversity. While increasing chemical defenses may depend on similar or shared compounds (e.g., polyketides, shikimates and phenylpropanoids) among species that reduce tissue loss (Coley et al., 1985), which also lead to lower endemism of phytochemicals in high elevations or latitudes. That is, species experiencing similar environmental conditions are likely to share phenotypic traits because of the process of environmental filtering (Cavender-Bares et al., 2004). While more natural enemies could drive divergent selection between related plants in low elevations or latitudes, leading to increasing chemical dissimilarity/endemism (Kursar et al., 2009). However, an empirical study conducted along an elevational gradient indicated that low elevation regions with stable habitats and higher biotic pressures favored increasing phytochemical diversity for protection (Defossez et al., 2021). But high elevation regions with less biotic pressures and heterogeneous microhabitats had lower overall phytochemical diversity but increased phytochemical endemism (Defossez et al., 2021; Volf et al., 2022). In general, the aforementioned studies were solely conducted in temperate mountains. The absence of comprehensive large-scale investigations (spanning different latitudes and climatic types, as exemplified by Sedio et al., 2018a) restricts our comprehension of phytochemical diversity or turnover patterns and their adaptations to abiotic and biotic pressures, particularly within the rhizosphere.

Moreover, phytochemicals were verified to promote multi-trophic species coexistence by maintaining biotic interactions (e.g., leaf-herbivore) (Kursar et al., 2009; Wang et al., 2022). However, the maintenance mechanism of below-ground biodiversity is less clear than that of above-ground (Xu et al., 2023; Zhang et al., 2023), which can be revealed from the below-ground biotic interactions (e.g., root-microorganism). Here, two opposite paradigms shed light on species coexistence based on chemical niche partitioning in fine roots. One hypothesis proposes that closely related plant hosts possess similar natural enemies, thus evolving similar chemistry (Berenbaum and 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. That is, enemies often infest chemically similar plants and impose selective pressures on plants to diverge chemically or bias community assembly toward chemical divergence (Becerra, 2007). The latter has been widely supported by recent studies in leaves, especially in tropics, i.e., the interactions of plants with their herbivores provide a high number of chemical niche dimensions and enhance species 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 is still largely unknown.

Due to the contrasting biotic and abiotic conditions along climatic gradients, the intensity of biotic interactions may be context-dependent (Schemske et al., 2009). For example, there are significant interactions between root chemistry and mycorrhizal fungi in resource-rich regions (Xia et al., 2021), but that in harsh regions showed no similar patterns (Spitzer et al., 2021). These observations may indicate the predictions of the Latitudinal Biotic Interaction Hypothesis (LBIH), which proposed that the intensity of biotic interactions increases from high to low latitudes (Schemske et al., 2009). A latitudinal gradient in the intensity of biotic interactions, to some degree, may explain the latitudinal pattern of biodiversity (Zvereva and Kozlov, 2021), i.e., the ubiquitous decrease in biodiversity from low to high latitudes (Lawton, 1999). 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 (Rasmann and Hiltpold, 2022). Based on LBIH, we 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 macroclimatic gradient spanning tropical, subtropical, and subalpine regions to explore the trends of complexity of phytochemical composition (e.g., alpha diversity) and the variation in composition (e.g., beta diversity or endemism) and their relationships with microorganisms along environmental gradients. To generate general or divergent trends of various metabolites, we analyze eight data subsets including total compounds and seven main metabolic pathways with different functions. We focus on the following questions: (i) What are the phytochemical diversity and endemism patterns of fine roots across climatic and elevational gradients? (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?

2. Materials and methods

2.1. Study sites and sampling procedures

In 2012, we established three field experimental platforms that span tropical (21°36′ N, 101°34′ E), subtropical (24°32′ N, 101°01′ E), and subalpine/temperate (hereafter "subalpine") (27°08′ N, 100°12′ E) climatic gradients (Fig. S1, Song et al., 2021), which represent a climatic/latitudinal gradient. The tropical platform represents montane rainforest ecosystems and includes four elevations: 800 m, 1000 m, 1200 m, and 1400 m. The subtropical platform represents mid-montane wet evergreen broad-leaved forest ecosystems and includes four elevations: 2000 m, 2200 m, 2400 m, and 2600 m. The subalpine platform represents temperate coniferous forest ecosystems that resemble the boreal forest of a temperate continental climate and includes four elevations: 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 (Xu et al., 2023; Zhang et al., 2023). Each elevation has five 20 m × 20 m plots for a total of 60 plots studied. The latitudinal span of three sites is about six degrees (ca. 600 km). 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; Xu et al., 2023). The analysis of each elevational gradient is conducted independently using standardized methods, and subsequently, the results obtained from the three climatic regions are compared.

The plots were evaluated for vegetation features, climatic variables (annual mean air temperature, temperature variability, annual mean air humidity, humidity variability and annual mean soil temperature), and soil properties (soil organic matter, total carbon, total nitrogen, total phosphorus, total potassium, available potassium, soil water content and soil pH) (details see Supporting Information 1, Zhang et al., 2023). To reduce the climate and soil data to a few major orthogonal axes of variability, a principal component analysis (PCA) was performed using the "FactoMineR" package (Sebastien et al., 2008). The first two PCA axes accounted for 94.82 % of the climate variation and 78.6 % of the soil variation were used for following analyses (Fig. S1). Elevational pattern of tree species in each climatic region was showed in Fig. S2. 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 in plots were sampled, then 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 of fine roots and rhizosphere soils 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.

2.2. Phytochemical metabolome measures

Metabolome in fine roots was extracted and analyzed following the procedure in Sedio et al. (2018a) with slight modification (also see Wang et al., 2022). The original UHPLC–MS/MS data were analyzed using the Global Natural Products Social (GNPS) Molecular Networking Metabolomics Tool (Wang et al., 2016) (details see Supporting Information 1). Based on the outputs of GNPS, we further annotated all compounds by classifying them according to the biosynthetic "pathway"-level classifications of NPClassiFier (Kim et al., 2021). The classification of these pathways includes: "Alkaloids", "Polyketides", "Shikimates and Phenylpropanoids", and "Terpenoids" that represent secondary metabolites, and "Amino acids and Peptides", "Carbohydrates", "Fatty acids" that represent primary metabolites. A small subset of compounds represents products of more than one major pathway (e.g., Alkaloids/Terpenoids). To address these cases, we followed the following principles: 1) if it is a compound that is classified into one secondary-metabolite category and one primary-metabolite category, it is classified into the secondary-metabolite category, 2) if the compound is classified into two secondary-metabolite categories, it is classified as "Multiple". Therefore, our final classification includes nine pathways, i.e., the aforementioned seven, "Multiple" and "Unknown". The functions of different pathways in plant defense were described in Supporting Information 1. We calculated two indexes, phytochemical diversity and phytochemical endemism of total compounds and seven pathways to represent the complexity and variability of phytochemicals, respectively. Phytochemical diversity was represented by the Shannon–Wiener diversity index (i.e., alpha diversity), which was calculated using the abundance matrix after standardized transformation (0–1) of plant compounds using the "vegan" package (Oksanen et al., 2015). Shannon diversity combines the richness and evenness of phytochemicals into a single metric, weighting the contribution of each compound such that compounds with lower abundances contribute less to the overall estimate of diversity, which could provide more ecologically relevant information of phytochemical complexity (Wetzel and Whitehead, 2020). Moreover, Shannon diversity also showed high correlations with chemical richness (Pearson's $r = 0.84, P < 0.001$) and abundance (Pearson's $r = 0.72, P < 0.001$). Based on the method of hill numbers (diversity order q) (Hsieh et al., 2016), Shannon diversity showed a good sampling coverage (Fig. S3). 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). Phytochemical endemism representing variability in phytochemistry is a key facet of diversity, which supplements the ecological connotation of phytochemical complexity (Wetzel and Whitehead, 2020). 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), which provides a chemically meaningful measure of structural variation in metabolite composition.

2.3. Rhizosphere microbiome measures

Rhizosphere soils were used to extract metagenomic DNA. 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 highly probable and probable guilds with an identified single trophic mode 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). Elevational patterns in different climatic regions of microbial diversity are showed in Fig. S3. 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., 2023) using the "betapart" package (Baselga et al., 2021).

2.4. Statistical analyses

All statistical analyses were conducted in R 4.1.2 (R Core Team, 2021). Our data are species level data, meaning that each tree species has a pair of compound and microbial or environmental data, and our main analysis is performed at the species level, in addition, the above data is further divided into subsets by climatic regions. 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. Elevational patterns of tree diversity showed inconsistent relationships with that of phytochemical diversity (Fig. S2), which could weaken the effects of different species richness on the patterns of phytochemical diversity. To further eliminate the disturbance of species richness and abundance on phytochemical diversity at the community level, we conducted the following analyses at the tree species level. 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 "coxme" package (Therneau, 2018). Phytochemical diversity was the response variable in the linear mixed effect models, climatic factors, soil properties, and microbial Shannon–Wiener diversity were predictor variables (fixed effects), and tree species ID were random effects, in which the phylogenetic distance matrix was treated as the variance family of random term that will account for the effects of phylogenetic relatedness. 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 and Qian, 2022). It is the largest dated tree of life for vascular plants and is widely used in ecology (Zhang et al., 2022). Phylogenetic distances between paired tree species were calculated using the "ape" package (Paradis and Schliep, 2018). Then, Mantel tests were used to test the relationships between phylogenetic distances and phytochemical similarities using the "vegan" package. For our phytochemical traits (i.e., endemism/dissimilarity), the number of trait dimensions always exceeds the number of species in a phylogeny (Wang et al., 2022). Thus, K_{mult} was calculated to evaluate phylogenetic signals in high-dimensional multivariate traits (Adams, 2014). Using random simulations based on Brownian motion, K_{mult} values <1 imply that the phenotypes of species resemble each other less than expected under Brownian motion, whereas K_{mult} values >1 imply that the phenotypes of closely related species are more similar to one another than expected under Brownian motion. Moreover, we also explored the relationships between microbial community dissimilarities (Bray–Curtis) and phytochemical endemism using partial Mantel tests by "vegan" package, in which a phylogenetic distance matrix was included for accounting for the effects of phylogenetic relatedness.

3. Results

3.1. Phytochemical diversity and endemism patterns of tree roots

In tropical regions, phytochemical diversity showed an obvious hump pattern along elevation (i.e., unimodal pattern at middle elevation) (Fig. 1a). In subtropical regions, phytochemical diversity also showed a hump pattern along elevation (Fig. 1a). In subalpine regions, phytochemical diversity showed a slight hump pattern along elevation (Fig. 1a). Diversity patterns of different pathways also showed hump patterns, especially in tropical and subtropical regions, except that Alkaloids and Polyketides increased with elevations in subalpine regions (Fig. S4). 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. 1c), 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 & c). The pathway diversity patterns between different climatic regions are generally similar to that of total compounds, especially for secondary metabolites (Fig. S5). The diversity proportions of different pathways in each elevation were showed in Fig. 1e.

In subalpine and subtropical regions, phytochemical endemism showed an obvious hump pattern along elevation, but phytochemical endemism showed a U-shaped pattern in the tropical region (Fig. 1b). For total compounds, subtropical regions had the lowest phytochemical similarity, followed by tropical regions, and subalpine regions had the highest phytochemical similarity (Fig. 1d). The similarity or endemism patterns of different pathways along climatic gradients were similar to that of total compounds (Fig. S6). 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. S7), which means that more closely related plant species showed higher phytochemical dissimilarity/endemism. Moreover, total 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 ($K_{mult} = 0.121$, $P > 0.05$). All pathways also showed no phylogenetic signals in any of the climatic regions (Table S2).

3.2. The relationships between phytochemical diversity and microbial diversity

For total compounds, total fungal diversity showed no significant relationship with phytochemical diversity in all cases (in total, tropical,

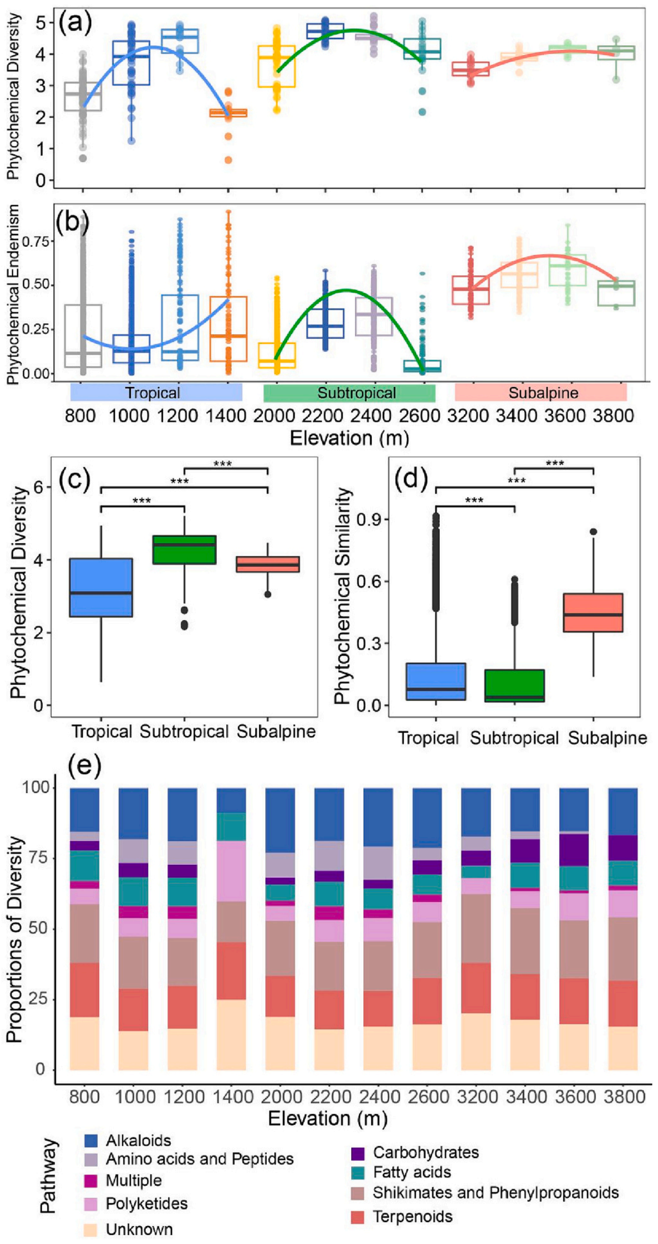

Fig. 1. Phytochemical diversity and endemism patterns across climatic gradients. (a) Phytochemical diversity patterns along elevation in different climatic regions. (b) Phytochemical endemism patterns along elevation in different climatic regions. Lines were fitted by multinomial linear regressions. (c) Phytochemical diversity difference between different climatic regions. (d) Phytochemical endemism difference between different climatic regions, i.e., low similarity means high endemism. (e) Proportions of pathways diversity in different elevations. ***$p < 0.001$.

subtropical and subalpine regions) ($P > 0.05$) (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 (i.e., three climatic regions together) ($R^2 = 0.21, P < 0.001$) (Fig. 2b). Total bacterial 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). 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 (i.e., R^2 quantified by hierarchical partitioning methods) of abiotic factors in total explained variance increased from tropical regions to subalpine regions (Fig. 3). In particular, climatic factors (including temperature and water conditions) were the most important predictor of phytochemical diversity in subalpine regions. For pathway diversity, climatic factors showed consistent significant effects in different climatic regions and for different pathways (Tables S3 & 4). For Shikimates and Phenylpropanoids, Terpenoids, Polyketides and Fatty acids diversity, fungal diversity showed significant effects in tropics (Table S3). For Alkaloids, Terpenoids, Shikimates and Phenylpropanoids, Fatty acids, and Amino acids and Peptides, bacterial diversity showed significant effects in tropics (Table S4).

3.3. The relationships between phytochemical endemism and microbial dissimilarity

For total compounds, total fungal 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 bacterial 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). For all secondary metabolites (Alkaloids, Terpenoids, Shikimates and Phenylpropanoids, and Polyketides) and Carbohydrates, the relationships between microbial dissimilarity and phytochemical endemism decreased from tropical to subalpine regions (Fig. S8). Fatty acids and Amino acids and Peptides endemism showed strong relationships to microbial dissimilarity in all climatic region (Fig. S8).

4. Discussion

4.1. 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., Inga and Piper) (Kursar et al., 2009; Richards et al., 2015) in a single community/plot (e.g., Euphorbiaceae in a plot) (Wang et al., 2022) or in a region (e.g., Inga across the Amazon) (Endara et al., 2021). Studies involving analyses of phytochemicals across climatic regions are uncommon due to difficult sampling and limited methods. A recent regional study focusing on grassland communities along an elevational gradient revealed the spatial predictability of foliar phytochemical diversity (Defossez et al., 2021). They found that low elevation habitats with less stressful abiotic

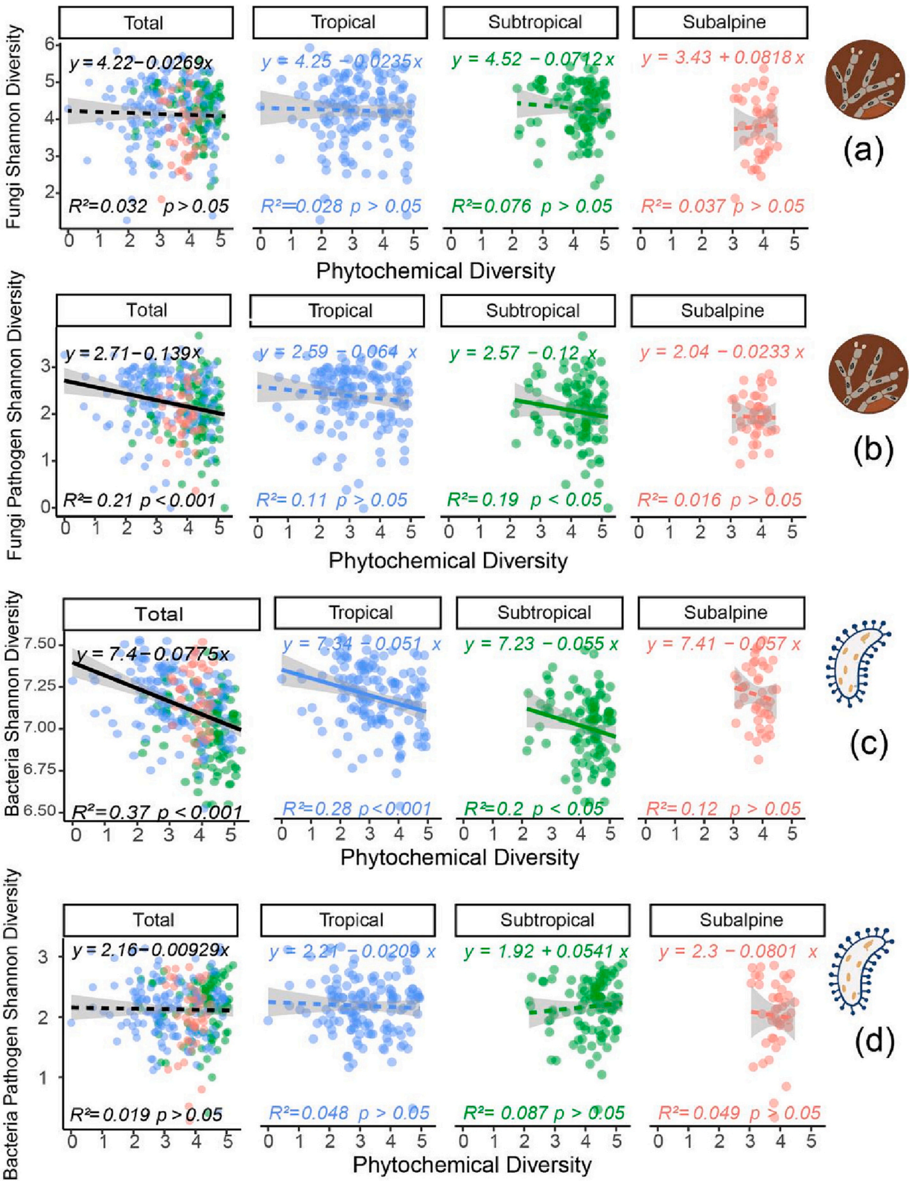

Fig. 2. The relationships between phytochemical diversity and microbial diversity in total climatic regions, tropical, subtropical, and subalpine regions, respectively. (a) Total fungi diversity. (b) Fungal pathogen diversity. (c) Total bacteria diversity. (d) Bacterial pathogen diversity. Lines were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

environments, but greater biotic pressures favor increased phytochemical diversity for protection (Coley and Aide, 1991), while high elevation regions with heterogeneous habitats favor decreased phytochemical diversity but select specific molecules essential for survival in stressful environments (i.e., higher phytochemical endemism) (Jacobo-Velázquez and Cisneros-Zevallos, 2018; Volf et al., 2022).

However, our study across large-scale climatic gradients and focused on fine roots revealed a different pattern between subalpine and tropical regions (excluding subtropical regions) (Fig. 1c), i.e., subalpine regions showed higher phytochemical diversity than tropical regions, especially for total compounds and some pathways (e.g., Alkaloids, Carbohydrates, and Shikimates and Phenylpropanoids). This result, i.e., higher chemical defenses at higher latitudes or elevations, is supported by previous studies (Moles et al., 2011), and noted as a positive adaptation to stressful environments (high ultraviolet, high wind, and freezing cold) (Rasmann et al., 2014; Volf et al., 2020). Thus, we found that many pathways (e.g., Alkaloids and Shikimates and Phenylpropanoids) increased with elevations in subalpine regions (Fig. S4), which may reflect their positive defense against high-elevation stresses (Korkina, 2007; Zhu et al., 2022). It is worth noting that tropical regions have higher variation in phytochemical diversity and greater phytochemical endemism for total compounds and various pathways, whereas subalpine regions have less variation and lower phytochemical endemism. Subalpine habitats have high abiotic stresses, such high UV irradiation and low temperatures, thus these harsh environmental pressures may lead to low phytochemical variation (environmental filtering for similar metabolites) (Cavender-Bares et al., 2004; Bakhtiari et al., 2021). 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.

We found an interesting phenomenon in mid-latitude regions, i.e., subtropical regions had the highest phytochemical diversity and

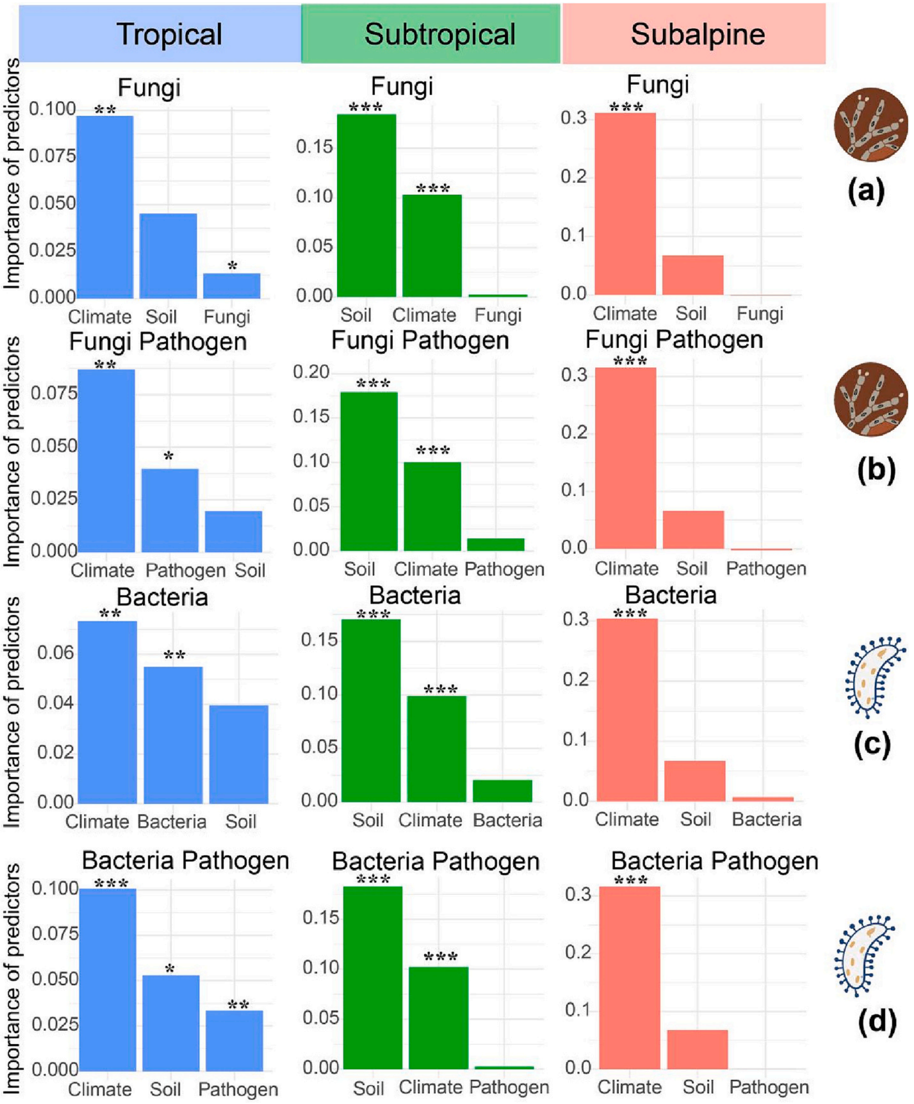

Fig. 3. The relative importance of predictors that shape phytochemical diversity in different climatic regions based on hierarchical partitioning methods. (a) Total fungi diversity. (b) Fungal pathogen diversity. (c) Total bacteria diversity. (d) Bacterial pathogen diversity. Phytochemical diversity 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. Fungi/bacteria/pathogen: the Shannon–Wiener diversity index of fungi, bacteria, and pathogen. *P* values were resulted from mixed effect models, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

endemism (Figs. 1c, d & S5). Previous studies indicates that intermediate levels of stress can promote investment in chemical defense, which may explain the hump-shaped elevational distribution of phytochemical (Sam et al., 2020; Volf et al., 2020). We assume that plants in subtropical regions need to cope with mixed pressure from both abiotic and biotic conditions, but tropical or subalpine species only cope with high biotic pressure and negligible abiotic pressures or stressful environments and negligible biotic pressures, respectively. Moreover, many pathways may have various ecological functions to defend both biotic and abiotic stresses (Supporting Information 1, e.g., Korkina, 2007; Vickers et al., 2009; Lim et al., 2017). Our results also found that the mid-regional pathogen communities are consisting of a mix of lowland and highland species (Fig. S9) and mid-regional tree species possess more unique and mixed compounds (Fig. S10). Thus, subtropical species should evolve to harbor two categories of phytochemicals to adapt to both abiotic and biotic pressures, higher phytochemical diversity and endemism. This explanation also fits with the hump pattern of phytochemical diversity along elevations in tropical and subtropical regions (Fig. 1a), here we term this pattern the “middle enrichment phenomenon”.

4.2. Root chemical niche partitioning promotes species coexistence

Higher phytochemical dissimilarity/endemism of total compounds and all pathways in the tropics than in the subalpine regions (Fig. 1d and Fig. S6) 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 (for all pathways) were not regulated by phylogeny. However, our findings contrast with Sedio et al. (2018b), who

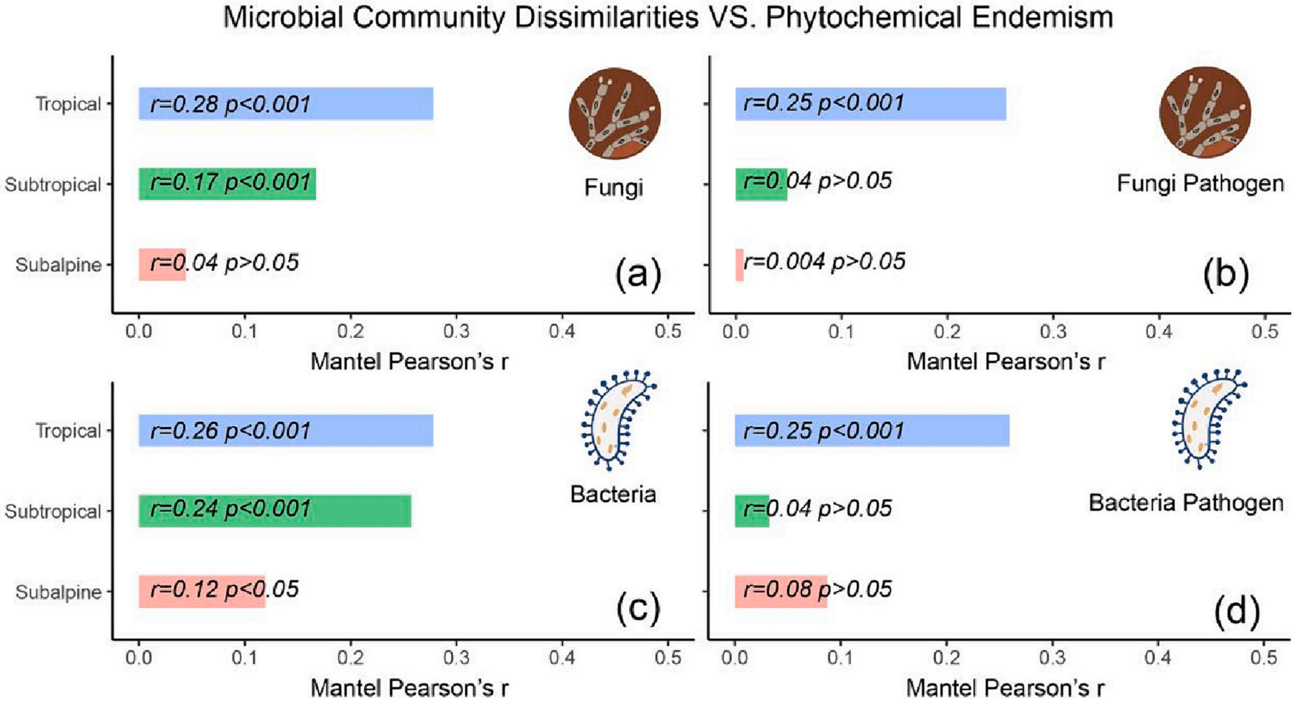

Fig. 4. The relationships between microbial community dissimilarities and phytochemical endemism in different climatic regions. (a) Total fungi dissimilarities. (b) Fungal pathogen dissimilarities. (c) Total bacteria dissimilarities. (d) Bacterial pathogen dissimilarities. Mantel Pearson's r and p value are given.

found that foliar phytochemicals were phylogenetically conserved for temperate species but not for tropical species. Importantly, our findings extend the universality of the chemical niche partitioning hypothesis in regional species pools beyond specific taxa (Kursar et al., 2009), and contrast with the prevailing coevolutionary theory: closely related plant species should be similar in defense strategies (Ehrlich and Raven, 1964). Surprisingly, all pathways (secondary or primary metabolites) showed obvious chemical niche partitioning, which suggests that total metabolite pool makes a collective contribution to species coexistence and not just due to specific metabolites. Moreover, species phylogeny alone poorly predicts phytochemical diversity, thus abiotic and biotic pressures should be considered in shaping phytochemicals (Defossez et al., 2021).

4.3. Phytochemical-microorganism interactions along climatic gradients and multi-trophic species coexistence

The Latitudinal Biotic Interaction Hypothesis (LBIH) suggests that the intensity of biotic interactions increases from high to low latitudes, which drives the biodiversity patterns along latitudinal gradients (Schemske et al., 2009). Our results found that the explanatory powers of phytochemical-microorganism models decrease from tropical to subalpine regions (especially for total compounds, Alkaloids, Terpenoids, Shikimates and Phenylpropanoids, Fatty acids, and Amino acids and Peptides), which supports the prediction of LBIH (Figs. 2 & 3). In contrast, although abiotic factors (especially climate) 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 (subalpine or temperate regions). That may explain why Volf et al. (2022) found a weak correlation of specialized chemistry with fungal damage and herbivory in a temperate mountain.

In addition to alpha diversity findings involved in the relationships between phytochemical and microbial diversity, we also provide similar evidence from beta diversity. 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 for total compounds, all secondary metabolites and some primary metabolites (e.g., Carbohydrates). 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). Thus, to some degree, the significant correlations between microbial dissimilarity (e.g., pathogens) and phytochemical endemism found in our study may indirectly reflect the relative intensity of biotic interactions. Thus, 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 (e.g., tropical regions), 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., 2023). However, some primary metabolites (e.g., Amino acids and Peptides, and Fatty acids) showed consistently strong interactions to microbial communities in different climatic regions, which indicates that biological interactions at high latitudes should be taken seriously in future. For example, it is important to increase anti-fungal defenses at high elevations, because the relative cost to replace lost tissue is greater in harsh environments (Volf et al., 2022).

5. Conclusions

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. We found higher variation in phytochemical diversity and higher phytochemical endemism in tropical tree species

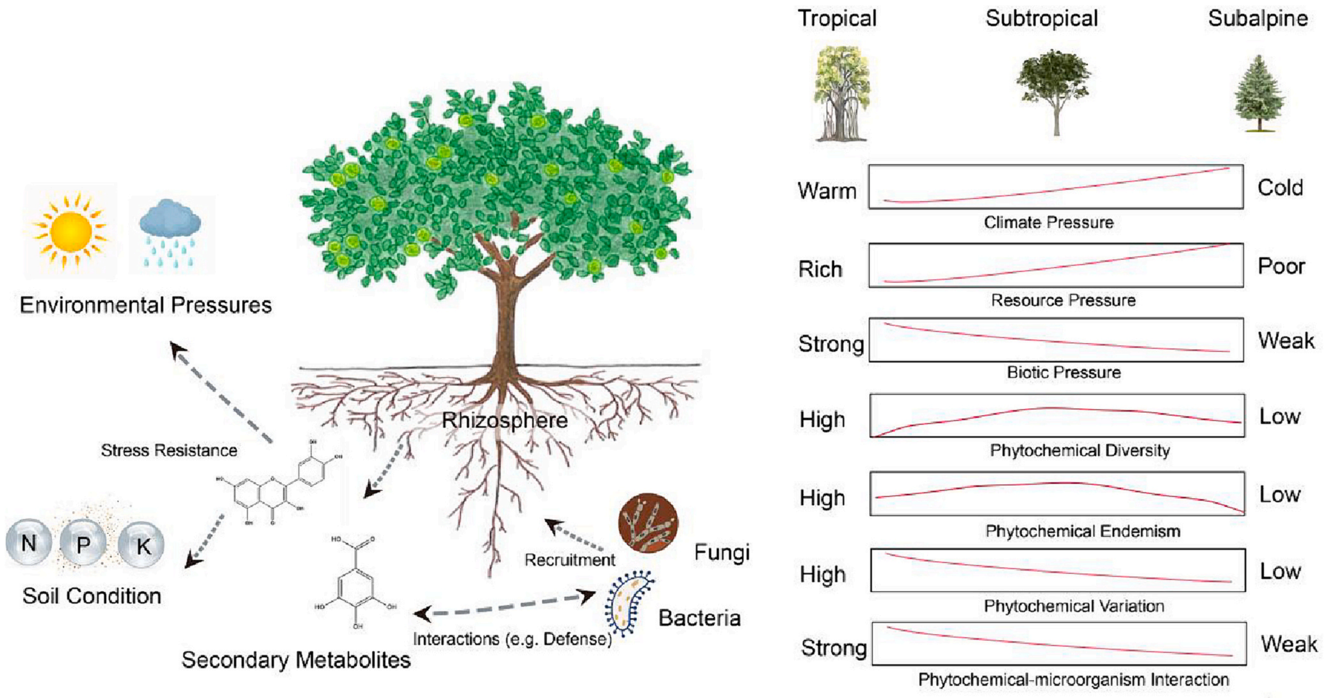

Fig. 5. Diagram of phytochemical patterns and their relationships with abiotic and biotic factors along climatic gradients. Climate and resource pressures reflect the stressful conditions from environments. Biotic pressure reflects the effects from natural enemies (e.g., herbivores and pathogens). Phytochemical variation reflects the total phytochemical difference between local species. Phytochemical endemism reflects the phytochemical dissimilarity between local species.

while subalpine tree species harbored higher phytochemical diversity but lower phytochemical variation and endemism, which may play important roles in coping with high biotic and abiotic pressures in tropical and subalpine regions, respectively (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 and phytochemical niche partitioning of host plants than that of higher latitudes, which shapes the enormous multi-trophic coexistence in the tropics. 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.

CRediT authorship contribution statement

Yazhou Zhang: Writing – original draft, Visualization, Methodology, Formal analysis. **Samantha J. Worthy:** Writing – review & editing. **Shijia Xu:** Methodology, Investigation. **Yunyun He:** Methodology. **Xuezhao Wang:** Methodology. **Xiaoyang Song:** Investigation. **Min Cao:** Investigation. **Jie Yang:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw microbial sequences are deposited in the National Genomics Data Center under Bioproject fungi CRA006600 and bacteria CRA006619. All methods and processes can be obtained directly from the online databases or publications described in the Methods, and Supporting Information.

Acknowledgment

This research was supported by the National Natural Science Foundation of China (32201315; 31870410); NSFC China-US Dimensions of Biodiversity Grant (DEB: 32061123003); the National Key R&D Program of China (2022YFF1302401); the Chinese Academy of Sciences Youth Innovation Promotion Association (Y202080); the Distinguished Youth Scholar of Yunnan (202101AV070005); the Ten Thousand Talent Plans for Young Top-notch Talents of Yunnan Province (YNWR-QNBJ-2018-309); the 14th Five-Year Plan of the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (XTBG -1450101); China Postdoctoral Science Foundation (2022M713343) and Postdoctoral Fellowship of Xishuangbanna Tropical Botanical Garden, CAS. We also thank Rensheng Zhao, Pengfei Song, Mufeng Cui, Lu Sun, Jie Wang and other crews for their contributions to the field and experimental work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.172051.

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