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Contrasting Patterns of Phyllosphere Fungal Diversity Between Angiosperms and Gymnosperms Along a Subalpine-Elevation Gradient

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ABSTRACT

Leaf functional traits play a fundamental role in shaping phyllosphere microbial diversity; however, their influence across evolutionary lineages and elevational gradients remains insufficiently understood. This study investigates the α-diversity, β-diversity, community assembly processes, and co-occurrence networks of phyllosphere fungi in Angiosperms and Gymnosperms along an elevational gradient (2900–4100 m) on Bai Ma Snow Mountain, southwestern China. By analyzing leaf functional traits and environmental factors, we evaluated their effects on fungal diversity, community assembly, and network stability under varying environmental conditions. Fungal α-diversity in Angiosperms followed a cosine-like pattern, peaking at mid-elevations, whereas Gymnosperms exhibited a consistent decline with increasing elevation. β-Diversity patterns revealed more pronounced structural shifts in Angiosperms, indicating stronger sensitivity to environmental gradients. Deterministic processes dominated community assembly at higher elevations, while stochastic processes were more influential at low-to-mid elevations, particularly in Angiosperms. Key leaf functional traits, including specific leaf area (SLA), leaf vein angle (LVA), and roughness (RS), were strongly correlated with fungal α-diversity in Angiosperms, with SLA and LVA showing negative correlations and RS a positive correlation. In contrast, Gymnosperms exhibited weaker associations with these traits. Co-occurrence network analyses revealed dynamic shifts in microbial connectivity in Angiosperms, where positive and negative interactions initially decreased but increased again at higher elevations. In contrast, Gymnosperms exhibited a steady decline in positive and negative interaction ratios along the gradient. These findings provide a foundation for integrating plant functional traits and microbial interactions into conservation strategies, prioritizing core fungal taxa, rare Angiosperms at higher elevations, and fragile alpine ecosystems, to enhance specific functional-taxa conservation and ecosystem resilience.

[Correction added on 18th August, 2025, after the first online publication: In Section 3.5, “R^2” has been corrected to “R” in the second and third paragraphs.]

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Summary

Plant leaves host diverse fungal communities that play a vital role in ecosystem health. This study investigated how leaf characteristics of different plant types (angiosperms and gymnosperms) influence fungal diversity along mountain elevations. We collected leaves from both angiosperms and gymnosperms at different elevations (2900–4100 m) in the Bai Ma Snow Mountain. We found that angiosperms showed more complex patterns of fungal diversity, with mid-elevation areas hosting the highest diversity, while gymnosperms showed a steady decline with increasing elevation. Leaf traits like specific leaf area, leaf vein angles, and leaf surface roughness strongly influenced fungal communities in angiosperms but had limited effects on gymnosperms. At higher elevations, angiosperms showed more dynamic fungal community structures, while gymnosperms maintained stable patterns. These findings highlight that by prioritizing the conservation of certain plant species and improving plant-microbe interactions, we can enhance the resilience of these fragile ecosystems.

- **Practitioner points**

  Based on this study's findings, we recommend three key conservation strategies:
  - Conservation of core fungal taxa and rare angiosperms with complex leaf traits (specific leaf area, roughness, and leaf vein angles) in high-elevation regions to enhance microbial habitat complexity and alpine ecosystem resilience.
  - Strengthening plant-microbe mutualisms through the use of biological inoculants at mid-to-high elevations, focusing on angiosperm species that sustain diverse fungal communities under environmental stress.
  - Implementation of leaf trait-based predictive models that integrate climate projections to guide dynamic conservation strategies. These models would facilitate selection of restoration species that maintain optimal microbial diversity across changing environmental gradients, thereby enabling targeted conservation efforts for ecosystem resilience.

1 | Introduction

Plant foliage, covering over 100 million square kilometers and hosting up to 10^{26} microbial cells, represents one of Earth's largest microbial habitats and contributes approximately 30 gigatons of biomass globally (Bar-On and Milo 2019; Zhu et al. 2022). Phyllosphere microbial communities, including bacteria and fungi, play pivotal roles in nutrient cycling, hormone regulation, and pathogen defense, thereby enhancing plant resilience to environmental stressors (He et al. 2005; Wang et al. 2023). Among these, fungi are particularly critical for organic matter decomposition, nutrient availability, and symbiotic associations that protect plants against pathogens and abiotic stresses such as temperature fluctuations and drought (Osono 2006; Izuno et al. 2016). Despite their ecological importance, these microbial communities face significant threats from rising temperatures, altered precipitation patterns, and increasing extreme weather events, which may compromise their stability and functionality (T. Yang et al. 2016). Positioned at the interface between plants and the atmosphere, phyllosphere microbes regulate microenvironmental conditions, facilitate nutrient exchange, and buffer against environmental fluctuations, underscoring their role in maintaining ecosystem resilience (Kembel et al. 2014). Understanding how microbial diversity, particularly fungi, responds to environmental gradients and ecological pressures is essential for informing conservation strategies that sustain biodiversity and ecosystem stability under accelerating global environmental change.

Mountain ecosystems, with steep environmental gradients across short geographic distances (Rahbek 1995), serve as natural laboratories for studying ecological dynamics and species adaptations (Bashir et al. 2022). These gradients, characterized by variations in temperature, humidity, and light availability, enable investigations into biodiversity patterns, functional traits, and community compositions across taxa, including microbes, insects, birds, and plants (Sanders 2002; Duclos et al. 2019; Le Bagousse-Pinguet et al. 2018). Elevational gradients often reveal shifts in species richness and microbial diversity, ranging from monotonic declines to hump-shaped or non-linear trends. The environmental filtering hypothesis suggests diversity typically decreases with elevation due to intensified stressors (Le Bagousse-Pinguet et al. 2018; Wang et al. 2023). While some studies observed monotonic declines (Wang et al. 2023), others reported sinusoidal patterns or strong influences of local factors, such as pH and nutrients, particularly for fungi (Kou et al. 2023; Siles and Margesin 2016), yet patterns remain inconsistent. Phyllosphere fungi, with their adaptability and functional diversity, are vital for enhancing plant resilience and maintaining ecosystem stability, making them essential for conserving biodiversity and supporting ecosystem restoration in climate-vulnerable mountain regions (T. Yang et al. 2016). Investigating the dynamic responses of phyllosphere microbes along elevational gradients provides crucial insights into ecosystem adaptability, offering a foundation for targeted conservation and restoration strategies to maintain biodiversity and ecological stability under environmental change, particularly for the relatively fewer Angiosperm species found at higher elevations in fragile alpine and subalpine ecosystems.

Angiosperms *and* Gymnosperms, two major evolutionary lineages of vascular plants, exhibit distinct morphological and functional traits shaped by divergent evolutionary paths, influencing their ecological interactions and responses to environmental changes (McElwain et al. 2016). Gymnosperms, which emerged earlier and dominated terrestrial ecosystems until Angiosperms diversified during the Late Cretaceous, evolved traits like lower specific leaf area (SLA), simpler venation, and thicker surfaces to withstand harsh, resource-limited environments (Bond 1989; Sack and Scoffoni 2013). In contrast, Angiosperms developed higher SLA and enhanced photosynthetic efficiency, enabling greater adaptability to diverse habitats and fluctuating conditions. These contrasting traits not only determine plant resilience under environmental stress but also mediate interactions with phyllosphere microbial communities, which support nutrient cycling, pathogen defense, and ecological stability (Luo et al. 2019; Chaudhry et al. 2020). However, whether the historical evolution of leaf traits influences microbial aggregation remains unclear. Clarifying how leaf traits shape microbial assembly is crucial for biodiversity conservation and developing adaptive strategies to mitigate the impacts of global change.

The leaf surface, or "microtopography," provides a critical habitat for microbial communities, with traits such as vein density, stomatal length, and surface roughness (RS) directly influencing microbial colonization and assembly (Yan et al. 2022). Angiosperms, characterized by higher SLA, denser venation, and rougher surfaces, promote greater microbial adhesion and diversity (McElwain et al. 2016), whereas Gymnosperms, with smoother surfaces and lower SLA, favor stress-tolerant taxa over diverse colonizers (Choat et al. 2012). These structural traits regulate water dynamics, nutrient availability, and microbial stability, shaping microbial composition and functional roles (Doan et al. 2020; Zhou et al. 2020). While bacterial colonization patterns have been extensively studied, the influence of leaf traits on phyllosphere fungi—essential for organic matter decomposition, nutrient cycling, and ecosystem stability—remains poorly understood (Osono 2006; Izuno et al. 2016). Addressing this knowledge gap is vital for advancing biodiversity conservation and enhancing ecosystem resilience, particularly in the face of global environmental change.

Here, we addressed the knowledge gap regarding phyllosphere fungi by exploring how leaf traits, such as SLA, roughness, and vein density, shape fungal diversity, community assembly, and co-occurrence networks. Leveraging the steep elevational gradient (2900–4100 m) of the Bai Ma Snow Mountain National Nature Reserve, we investigate the influence of plant functional traits and environmental factors on fungal community dynamics. Specifically, we aim to address three key scientific questions: (1) Do phyllosphere fungi exhibit similar decline diversity patterns with elevation increased as shown in previous studies, regardless of whether they are Angiosperms or Gymnosperms? If no, (2) What are the differences in fungal diversity between evolutionary host plant lineages—Angiosperms and Gymnosperms, and are they driven by variations in leaf traits? Furthermore, (3) How do leaf traits influence community assembly and network stability between these lineages? Addressing these questions provides insights into how plant functional traits and microbial interactions shape phyllosphere fungal communities along environmental gradients, thereby supporting adaptive conservation strategies aimed at enhancing ecosystem resilience and informing long-term protection measures for sensitive mountain environments.

2 | Materials and Methods

2.1 | Study Site

The research was conducted in the Bai Ma Snow Mountain National Nature Reserve (28°22′ N, 99°08′ E) in Yunnan Province, China (Figure 1b). This protected subalpine forest, located within the Diqing region on the northwestern edge of a globally recognized biodiversity hotspot, harbors numerous endemic and rare species. The reserve spans an elevation range of 2100–4200 m, encompassing steep gradients and distinct ecological zones. The region experiences a temperate montane monsoon climate influenced by seasonal monsoon patterns (Y. Yang et al. 2016).

The study area's elevation gradient ranges from 2900 m, dominated by *Huashan pine* forests, to 4100 m near the summit, where *larch* and *fir* species prevail. At higher elevations, Gymnosperms from the family *Fagaceae*, including *alpine pine*, *alpine oak*, *yellow-backed oak*, and *Huashan pine*, dominate the landscape. To minimize human disturbance at lower elevations (Figure 1a), sampling sites were carefully selected to ensure consistency in host plant species across the gradient. The pronounced climate variability and associated plant trait adaptations along the elevation gradient create diverse fungal communities, transitioning from forests to meadows and alpine landscapes. These distinct vegetation types provide a microcosmic perspective on the transition from subtropical to subalpine ecosystems.

2.2 | Experimental Design and Sampling

Fieldwork was conducted on Bai Ma Snow Mountain, where common host plants were selected across various elevations to

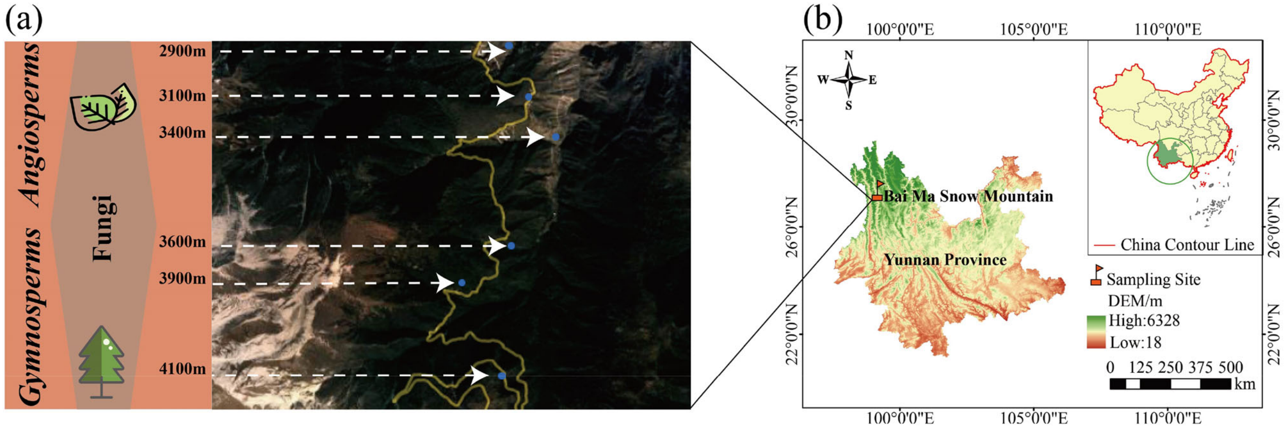

FIGURE 1 | Sampling diagram. Sampling sites across Bai Ma Snow Mountain. (a) Map of sampling locations in Yunnan Province, southwestern China. Samples were classified into two evolutionary groups—Angiosperms and Gymnosperms—based on phyllosphere fungi. (b) The subalpine region, including sampling plots at elevations of 2900, 3100, 3400, 3600, 3900, and 4100 m. Blue dots (a) represent sampling locations.

ensure a balanced representation of Angiosperms and Gymnosperms at each elevation. A total of 25 species spanning two evolutionary lineages (e.g., Angiosperms vs. Gymnosperms) were identified (De La Torre et al. 2017) through the Flora of China database (http://www.iplant.cn/foc). At each plot, 36–42 trees with a diameter at breast height (DBH) ≥ 5 cm were tagged for sampling.

Leaf samples were collected in September 2023 at six elevations (2900, 3100, 3400, 3600, 3900, and 4100 m), covering all major forest zones. At each elevation (i.e., each site), six sampling points within 100 × 100 m monitoring plots were established within 100 × 100 m dynamic monitoring plots collaboratively set up by the School of Ecology and Environmental Science, Yunnan University, and the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. Sampling points were spaced at least 50 m apart to minimize spatial autocorrelation.

Healthy, mature leaves were collected from six individuals of the same species at each sampling point. Samples were pooled into composite samples by species. Due to variations in dominant species across elevations (42, 36, 36, 36, 42, and 36 samples, respectively), the total number of samples differed across sites (Supporting Information S1: Table S1). Leaves free of visible disease or herbivory were collected from adult trees of each dominant species. Approximately 100 g of healthy leaves were divided into two subsamples—one stored at 4°C for leaf functional trait analysis and the other transported on dry ice and stored at −80°C for DNA extraction. In total, 456 leaf samples were collected from 38 tree species ($n = 228$), with some species distributed across multiple elevations.

2.3 | Leaf Functional Traits

We measured three common leaf functional traits per sample to capture variations related to resource allocation, stress responses, and leaf morphology, following established protocols. SLA reflects light interception efficiency and trade-offs between tissue longevity and construction costs. Leaf RS characterizes the microscopic and macroscopic unevenness of leaf surfaces, influencing microhabitat heterogeneity. Leaf vein angle (LVA), defined as the average angle between the main vein and lateral veins, describes resource allocation and structural organization (Y. Yang et al. 2016).

Leaf fresh mass (LFM) was determined using a precision balance, with six intact leaves randomly selected per species. An EPSON GT-20000 scanner was used to digitize leaf images, and leaf area (LA) was calculated using Image-Pro Plus 6.0 software (Media Cybernetics). SLA was calculated by dividing LA by LFM. Vein angles were measured using digital angle measurement tools, while RS was quantified using the same software (Wang et al. 2015). These traits provide insights into leaf adaptations and their influence on microbial colonization.

2.4 | DNA Extraction and Bioinformatics Analysis

Phyllosphere microbes, including both epiphytes and endophytes, were collected following the CTAB protocol (Yao et al. 2020). Fresh leaf samples (5 g) were washed in sterile, cooled TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) using ultrasonication for 1 min, followed by two 10-s vortex cycles (Kembel et al. 2014). Leaves were then ground in liquid nitrogen, and 5 g of leaf powder was used for DNA extraction (Bodenhausen et al. 2013). The washed samples were filtered through a 0.2 μm sterile membrane, and the membrane was stored at −80°C for further analysis (Herrmann et al. 2021).

Total genomic DNA was extracted from the frozen leaf samples using the FastDNA SPIN Kit (MP Biochemicals, Solon, OH, USA) following the manufacturer's instructions. The quality and quantity of the extracted DNA were checked via 1% agarose gel electrophoresis (Kembel and Mueller 2014). DNA concentration and purity were measured using a NanoDrop ND-2000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

The fungi ITS1 region was amplified by PCR using primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) (Gao et al. 2022). PCR reactions were carried out in a 25 μL mixture containing 5 μL GC buffer, 5 μL of 5× reaction buffer, 2 μL DNA template (0.2 ng/μL), 1.0 μL of each primer (10 μM), 2 μL of 2.5 mM dNTP, 0.25 μL of Q5 DNA Polymerase (New England Biolabs), and 8.75 μL of dd H_2O (Wang et al. 2023). The PCR conditions were as follows: initial denaturation at 98°C for 2 min; 30 cycles of 98°C for 15 s, 55°C for 30 s, and 72°C for 30 s; followed by a final extension at 72°C for 5 min. The PCR products were extracted from 2% agarose gel, purified using the QIAamp DNA Micro Kit (Qiagen, Valencia, CA, USA), and quantified with a Quantus Fluorometer (Promega) (Gao et al. 2022). Sequencing libraries were prepared using the TruSeq Nano DNA LT Library Prep Kit (Illumina, USA), and the quality of the constructed libraries was assessed using an Agilent Bioanalyzer. The final libraries were sequenced on the Illumina MiSeq. 250 platform by Shanghai Personal Biotechnology Co. Ltd. (China) (Gao et al. 2022).

2.5 | Climate Data Collection

Environmental parameters such as air temperature (AT), relative humidity (RH), photosynthetically active radiation (PAR), wind speed (WS), and soil electrical conductivity (SEC) were continuously monitored across elevations using meteorological stations installed by the School of Ecology and Environmental Science, Yunnan University (INTELL-SUN, Beijing Zhiyang Technology Co. Ltd.). Each elevation site was equipped with a data logger (CR1000X), barometric pressure sensor (CS106), photosynthetically active radiation sensor (LI-190), air temperature and humidity sensor (HMP60), wind speed and direction sensor (WindSonic), rain gauge (TE525), and soil parameter sensor (SoilVUE10-05). To consider the continuity and timeliness of data monitoring at different elevations in the rainy season, this study only used three environmental factors: AT, RH, and PAR (Supporting Information S1: Figure S1).

2.6 | Data Analysis and Visualization

Microbiome bioinformatics analysis was conducted using GenesCloud (www.genescloud.cn), provided by Personal

Biotechnology Co. Ltd. (Shanghai, China). Paired-end reads were demultiplexed using unique barcodes, followed by the removal of barcode and primer sequences. Raw reads were quality filtered using fqtrim (v0.94) to obtain high-quality clean tags, and chimeric sequences were removed. And chimeric sequences were removed. Amplicon sequence variants (ASVs) and feature tables were generated using DADA2 (Callahan et al. 2016). The Shannon-Wiener index was calculated using QIIME2 to evaluate the α-diversity of phyllosphere fungal communities, and the Kruskal-Wallis rank-sum test was used to assess differences across elevations (2900–4100 m) with statistical significance set at $p < 0.05$ (Bolyen et al. 2019). Principal coordinates analysis (PCoA) based on Bray-Curtis distance was conducted to examine microbial community differences, with additional analyses, including analysis of similarity (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) (Dixon 2003), performed using the "vegan" package in R to evaluate dissimilarities across the elevation gradient (Guo et al. 2018).

A null model-based approach was applied to quantify the contributions of stochastic and deterministic processes to community assembly. The normalized stochastic ratio (NST) was used to determine the relative contributions of these processes, where NST > 50% indicated stochastic dominance and NST < 50% indicated deterministic dominance (Ning et al. 2020). Modified stochastic ratios (MST) were calculated using the "NST" package in R. To test the effects of evolutionary history and elevation on MST, linear mixed models were constructed with plant species as a random effect and elevation and evolutionary history as fixed effects (Nestler 2022). Factor significance and interactions were assessed by comparing models with and without specific terms using the "lme4" package in R, with significance set at $p < 0.05$ (Lu et al. 2020). Visualizations were created using the "ggplot2" package, while maps were produced in ArcGIS 10.6 (ESRI, USA).

Microbial co-occurrence networks were constructed and visualized using the R packages "psych," "igraph," "ggraph," and "tidygraph" (Deng et al. 2016). We calculated OTU relative abundances and selected the top 20 most abundant taxa at each elevation (Barberán et al. 2014). Spearman correlation analysis identified significant relationships between taxa $|r| > 0.3$ and $p < 0.05$, with microbial taxa represented as nodes and edges indicating positive (red) or negative (blue) correlations. Edge widths corresponded to $|R|$, and node size reflected degree centrality. Networks were visualized using circular layouts, with nodes colored by genus and unclassified taxa grouped as "unclassified." Topological properties such as degree and clustering coefficients were calculated, and elevation-specific networks were combined for comparative analysis using the "cowplot" package (Yuan et al. 2021).

Mantel tests were conducted with the "linkET" and "vegan" packages in R to explore correlations between microbial α-diversity, β-diversity, network stability, and community assembly, as well as leaf functional traits, and environmental factors, using Spearman's correlation with a significance threshold of $p < 0.05$. Random Forest (RF) models ("randomForest" and "rfPermute" packages, R) were used to identify key leaf traits and environmental factors influencing fungal α-diversity. Feature importance was evaluated based on %IncMSE, with significance levels annotated as $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$ in bar plots generated with "ggplot2."

To investigate the relationship between fungal α-diversity and leaf traits (SLA, RS, and LVA) across elevations, linear regression models ("lm" function in the "stats" package, R) were applied. Elevation-specific slopes for fungal α-diversity in Angiosperms and Gymnosperms were also calculated using linear regression. Scatterplots with fitted regression lines were generated using the "ggplot2" package, with regression outputs including R^2 and p-values reported to quantify trait-diversity relationships. All analyses were conducted in R 4.4.1 software (R Core Team, 2022; http://www.r-project.org/).

3 | Results

3.1 | Patterns of Fungal Diversity and Leaf Traits at Different Elevations

The α-diversity of phyllosphere fungi in Angiosperms and Gymnosperms exhibited distinct elevation-dependent patterns, as measured by the Shannon index. In Angiosperms, the fungal α-diversity followed a cosine-like trend along the elevation gradient, showing no significant overall linear change (Figure 2; **slope = 0.002**). In contrast, Gymnosperms displayed a significant decline in fungal α-diversity with increasing elevation (Figure 2; **slope = −0.145**). Fungal α-diversity in Gymnosperms was significantly lower at higher elevations compared to lower ones (Figure 2; $\boldsymbol{p < 0.001}$). Notably, between 3400 and 4100 m, Angiosperms exhibited significantly higher fungal α-diversity than that observed in Gymnosperms (Figure 2a,b; $\boldsymbol{p < 0.05}$).

Overall, both plant lineages exhibited significant microbial community divergence along the elevation gradient (Supporting Information S1: Table S2; **PERMANOVA**), with Angiosperms showing more pronounced differentiation. Principal coordinate analysis (PCoA) based on Bray-Curtis distance matrices revealed significant elevation-driven differences in the fungal β-diversity for both Angiosperms and Gymnosperms. In Angiosperms, fungal communities exhibited pronounced structural differentiation along the elevation gradient, with distinct shifts between low elevations (2900 m) and mid-to-high elevations (3600–4100 m) (Supporting Information S1: Figure S2a; **PERMANOVA**; $\boldsymbol{p < 0.001}$). In contrast, Gymnosperms displayed greater overlap in fungal community structures at mid-to-low elevations but showed more notable divergence at higher elevations (Supporting Information S1: Figure S2b; **PERMANOVA**; $\boldsymbol{p < 0.001}$).

Distinct elevational patterns in leaf functional traits—SLA, RS, and LVA—were also observed between Angiosperms and Gymnosperms (Figure 3). SLA decreased with elevation in both groups; however, Angiosperms consistently maintained significantly higher values, whereas Gymnosperms showed a steeper decline (Figure 3a; $\boldsymbol{p < 0.05}$). RS patterns revealed contrasting trends, with Angiosperms displaying cosine-like fluctuations, peaking at 3400 and 4100 m, where RS values were significantly higher than those in Gymnosperms. In contrast, Gymnosperms demonstrated a steady and significant decline in RS with

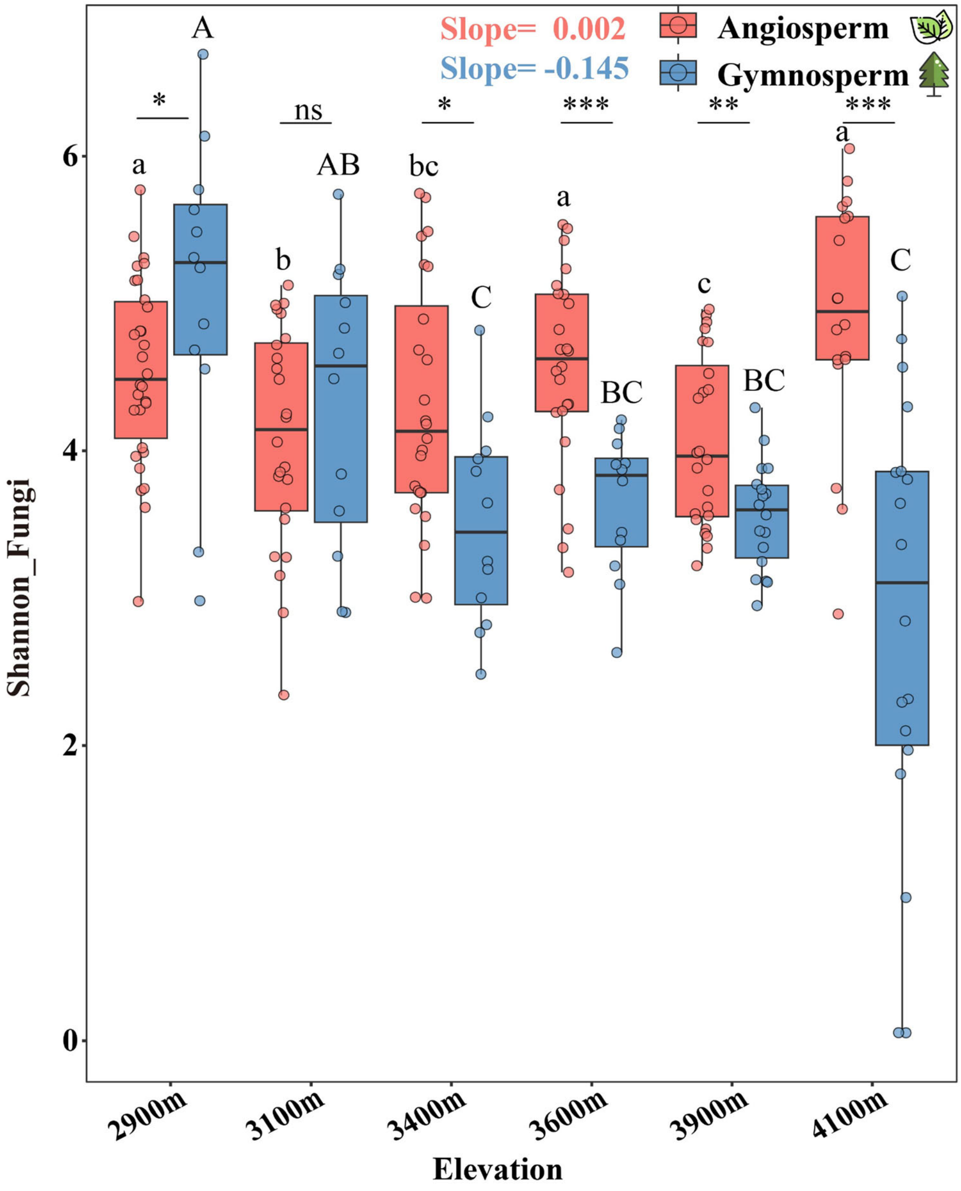

FIGURE 2 | The α-diversity patterns of fungal communities in Angiosperms and Gymnosperms. The α-diversity of phyllosphere fungal communities in Angiosperms and Gymnosperms across six elevations (2900, 3100, 3400, 3600, 3900, and 4100 m). Bars represent mean ± SD, and statistical α-diversity differences of Angiosperms and Gymnosperms were determined using Kruskal-Wallis rank-sum tests ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $^{ns}p > 0.5$). The lowercase and uppercase letters represent the significant differences between the two different historical evolutionary groups at six elevations.

increasing elevation (Figure 3b; $\boldsymbol{p < 0.05}$). Similarly, LVA remained relatively stable in Gymnosperms across elevations, while Angiosperms exhibited a significant decrease at higher elevations (Figure 3c; $\boldsymbol{p < 0.05}$). Notably, Angiosperms displayed significantly higher LVA at 2900–3100 m elevations but displayed significantly lower values than Gymnosperms at 3900–4100 m (Figure 3c; $\boldsymbol{p < 0.05}$).

3.2 | Fungal Community Composition at Different Elevations

Rarefaction curves of fungal communities in Angiosperms and Gymnosperms across elevations (2900–4100 m) demonstrated increasing species richness with sampling effort, eventually approaching saturation, indicating adequate sampling depth (Supporting Information S1: Figure S3). Angiosperms consistently exhibited higher species richness compared to Gymnosperms across all elevations (Supporting Information S1: Figure S3a,b).

Across the elevation gradient, the dominant phyllosphere fungal phylum in both Angiosperms and Gymnosperms was *Ascomycota*, with its relative abundance peaking at the highest elevation (4100 m) (Supporting Information S1: Figure S4a,b). In Angiosperms, the relative abundances of *Ascomycota*, *Basidiomycota*, and *Chytridiomycota* remained relatively stable across mid-elevations (3100–3900 m), whereas Gymnosperms displayed greater variability. Notably, the relative abundance of *Basidiomycota* was consistently more abundant in Angiosperms than in Gymnosperms across all elevations (Supporting Information S1:

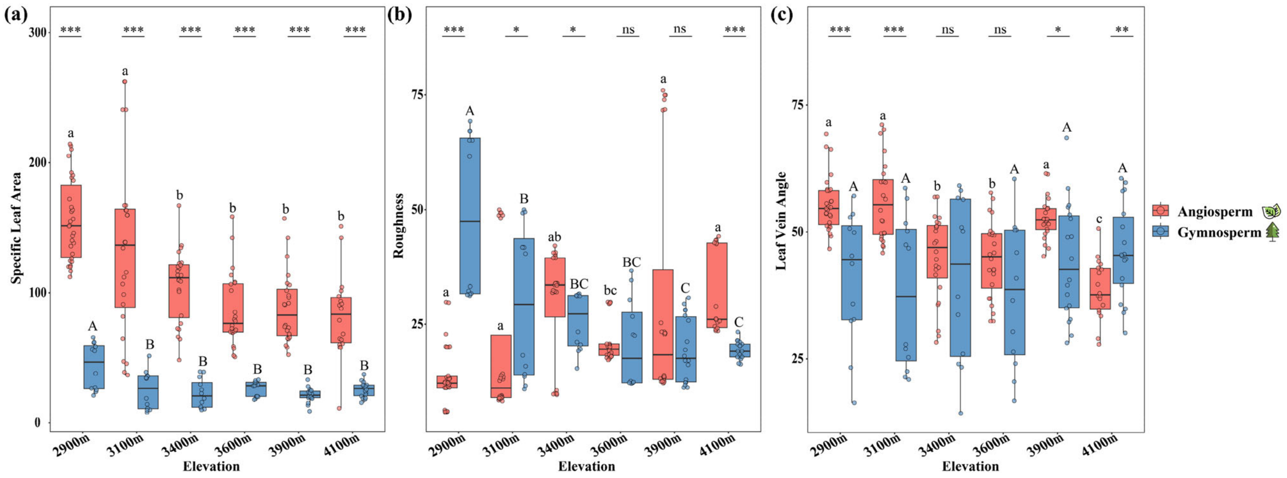

FIGURE 3 | Variation of leaf functional traits of Angiosperms and Gymnosperms along an elevation gradient. The leaf functional traits: specific leaf area (a), leaf roughness (b), leaf vein angle (c) of Angiosperms and Gymnosperms across six elevations (2900, 3100, 3400, 3600, 3900, and 4100 m). Bars represent mean ± SD, and statistical α-diversity differences of Angiosperms and Gymnosperms were determined using Kruskal-Wallis rank-sum tests ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{ns}p > 0.5$). The lowercase and uppercase letters represent the significant differences between the two different historical evolutionary groups at six elevations.

Figure S4a,b). For *Chytridiomycota*, Angiosperms exhibited higher relative abundances at low elevations (2900–3400 m) and at the highest elevation (4100 m), in contrast to Gymnosperms, where *Chytridiomycota* abundance surpassed *Basidiomycota*, ranking second only to *Ascomycota*. Furthermore, Angiosperms also maintained more stable relative abundances of *Ascomycota*, *Basidiomycota*, and *Chytridiomycota* across mid-elevations (3100–3900 m) compared to Gymnosperms. Collectively, *Ascomycota*, *Basidiomycota*, and *Chytridiomycota* accounted for over 75% of the total fungal communities in both groups, underscoring their dominant roles in shaping phyllosphere fungal diversity along the elevation gradient.

3.3 | Assembly of Phyllosphere Fungal Communities Along Elevation Gradients

To investigate the ecological processes driving community assembly, we applied the Sloan neutral model and calculated the MST to quantify the relative contributions of deterministic and stochastic processes in the phyllosphere fungal communities of Angiosperms and Gymnosperms (Supporting Information S1: Figure S5). The MST values indicated that deterministic processes predominantly governed fungal community assembly across all elevations, with their influence increasing at higher elevations. However, Angiosperms exhibited greater susceptibility to stochastic processes, as evidenced by significantly higher stochastic contributions across most elevations compared to Gymnosperms (Supporting Information S1: Figure S5; $\boldsymbol{p < 0.05}$).

The linear mixed-effects model further revealed a significant interaction between evolutionary history and elevation (Taxa×Elevation) in influencing MST values (Supporting Information S1: Table S3; $\boldsymbol{p < 0.001}$). While evolutionary history alone affected MST values, its influence aligned with the dominance of deterministic processes, particularly in Gymnosperms. In contrast, Angiosperms demonstrated a higher relative contribution of stochastic processes, emphasizing distinct community assembly dynamics between the two evolutionary lineages.

Overall, deterministic processes dominated fungal community assembly along the elevation gradient, with Gymnosperms exhibiting stronger deterministic structuring, while Angiosperms displayed greater stochasticity. These findings highlight the contrasting mechanisms governing fungal community assembly in Angiosperms and Gymnosperms, shaped by their evolutionary histories and responses to elevational gradients.

3.4 | Co-Occurrence Patterns of Phyllosphere Fungal Communities Along Elevation Gradients

The co-occurrence networks of phyllosphere fungi exhibited distinct elevation-driven patterns between Angiosperms and Gymnosperms (Supporting Information S1: Table S4). In Angiosperms, network complexity initially decreased with elevation but increased significantly at higher elevations (3900–4100 m). This shift corresponded to dynamic changes in the ratio of positive to negative interactions, which first declined and then gradually increased with elevation. At higher elevations, the rise in positive interactions enhanced network connectivity; however, overall connectivity remained lower than that observed at mid-elevations (3100–3400 m), reflecting a dynamic adaptation of Angiosperm fungal networks to environmental changes along the gradient (Supporting Information S1: Table S4).

In contrast, the fungal networks of Gymnosperms exhibited a steady increase in network complexity with elevation, as evidenced by a rising number of nodes and edges. Despite this structural expansion, the ratio of positive to negative interactions consistently decreased with elevation, resulting in weaker overall connectivity at higher elevations. This pattern highlights the distinct ecological responses of Gymnosperm fungal

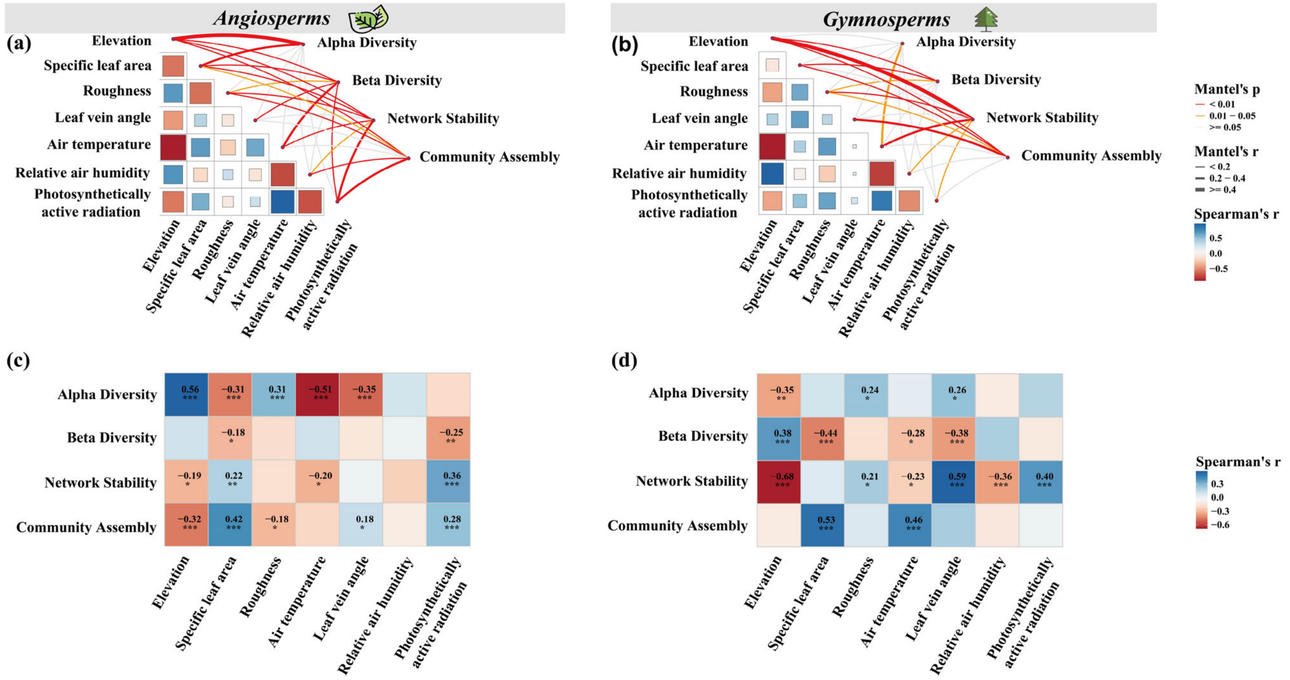

FIGURE 4 | Mantel test (MT) analysis and Spearman's correlation for fungal communities in Angiosperms (a, c) and Gymnosperms (b, d). MT analyzed the relationships between host plant functional traits, environmental factors, microbial diversity, community assembly, and network stability across elevations (2900, 3100, 3400, 3600, 3900, and 4100 m). The thickness of the lines represents the magnitude of Mantel's *r*-value of the correlation coefficient, while edge coloring indicates the level of statistical significance. Spearman's correlation between the microbial diversity, community assembly, and network stability, and environmental factors, including AT: air temperature, RH: air relative humidity, PAR: photosynthetically active radiation. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{\mathrm{ns}}p > 0.5$ means (not significant). Alpha diversity: Shannon Index.

networks to environmental stress at higher elevations (Supporting Information S1: Table S4).

Overall, these findings emphasize significant differences in elevation-driven patterns between Angiosperm and Gymnosperm fungal networks. While Gymnosperms demonstrated a gradual decline in positive interaction ratios, Angiosperms showed a more variable response, with positive interaction ratios initially decreasing before increasing at higher elevations.

3.5 | Factors Influencing Microbial Communities

Fungal α-diversity in both Angiosperms and Gymnosperms was significantly influenced by Elevation, RS, and LVA (Figure 4). Mantel test results revealed significant strong correlations between leaf traits, environmental factors, and fungal communities in both Angiosperms and Gymnosperms (Figure 4a,b; $\boldsymbol{p < 0.05}$). In Angiosperms, elevation and SLA were highly correlated with α-diversity, β-diversity, network stability, and community assembly processes (MSTs) (Figure 4a; $\boldsymbol{p < 0.05}$). Similarly, in both Angiosperms and Gymnosperms, elevation and leaf RS were strongly associated with β-diversity, network stability, and MST (Figure 4a,b; $\boldsymbol{p < 0.05}$).

Spearman's correlation analysis further indicated that fungal α-diversity, β-diversity, network stability, and MST in Angiosperms were significantly influenced by Elevation and SLA. Specifically, Elevation exhibited a significant positive correlation with α-diversity ($R = 0.56$) but a negative correlation with network stability ($R = -0.19$) and MST ($R = -0.32$). SLA exhibited a negative correlation with α-diversity ($R = -0.31$) and β-diversity ($R = -0.18$), while it was positively associated with network stability ($R = 0.22$) and MST ($R = 0.42$) (Figure 4c,d; $\boldsymbol{p < 0.05}$).

In Gymnosperms, α-diversity, β-diversity, and network stability were significantly correlated with Elevation and LVA. Elevation showed a significant negative correlation with α-diversity ($R = -0.35$) and network stability ($R = -0.68$), but a positive correlation with β-diversity ($R = 0.38$). Similarly, LVA was positively correlated with α-diversity ($R = 0.26$) and network stability ($R = 0.59$) but negatively correlated with β-diversity ($R = -0.38$).

RF analysis identified key drivers shaping phyllosphere fungal α-diversity of phyllosphere in Angiosperms and Gymnosperms (Figure 5a,b). In Angiosperms, α-diversity was primarily influenced by elevation (56.51%, $p < 0.01$), followed by LVA (18.76%, $p < 0.05$), SLA (16.26%, $p < 0.05$), RS (15.36%, $p < 0.05$), and AT (14.95%). In contrast, environmental variables such as PAR and RH had weaker impacts. For Gymnosperms, RS emerged as the most significant factor (9.22%, $p < 0.05$), followed by elevation (8.83%), AT (8.59%), and RH (4.98%). SLA (4.39%) and PAR (3.95%) contributed less, while LVA (−3.35%) had a negative effect. Notably, elevation was the dominant driver for fungal α-diversity in Angiosperms, whereas RS played a dominant role in Gymnosperms. Although both lineage groups were sensitive to RS, Gymnosperms exhibited greater sensitivity to environmental factors such as AT and RH.

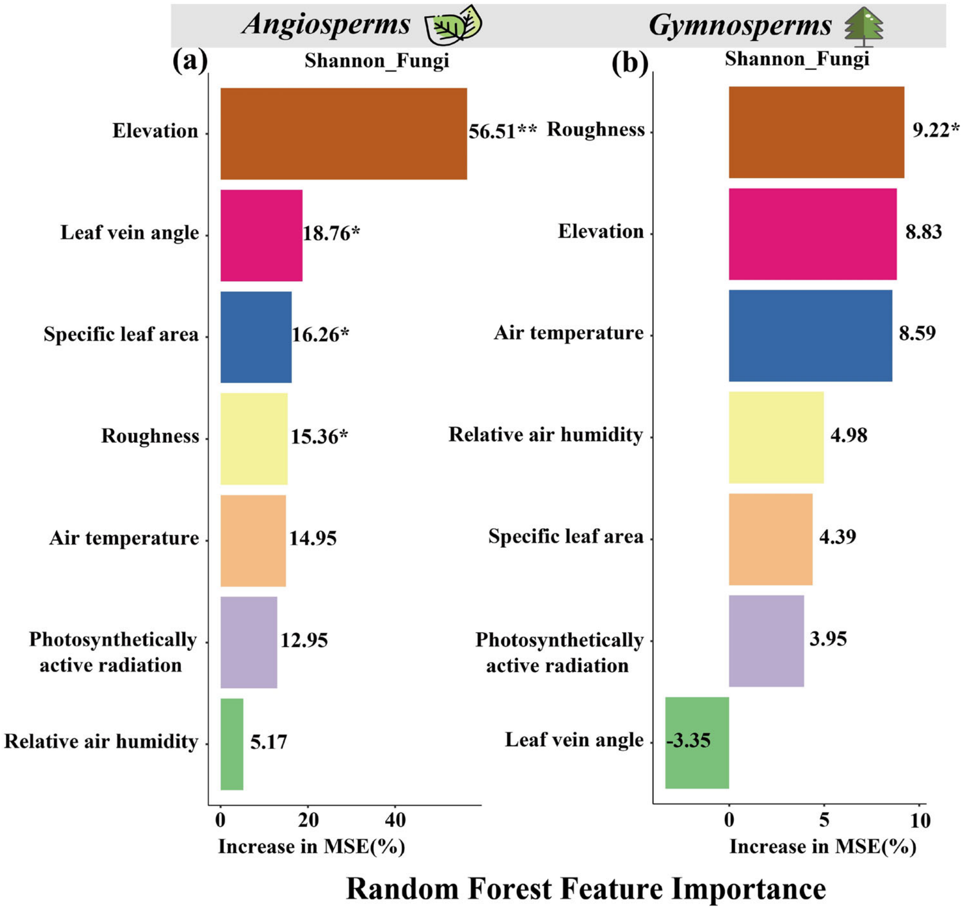

FIGURE 5 | Random Forest (RF) importance ranking for fungal communities in Angiosperms (a) and Gymnosperms (b). RF analysis determined the importance of host plant functional traits and environmental factors in the α diversity of phyllosphere fungal communities. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{\mathrm{ns}}p > 0.5$ means (not significant).

3.6 | Factors Influencing Fungal α-Diversity

The relationship between phyllosphere fungal α-diversity and key leaf traits—SLA, RS, and LVA across different elevations was evaluated using a linear regression model. In Angiosperms, fungal α-diversity exhibited a significant negative correlation with SLA and LVA (Figure 6a,c; $\boldsymbol{p < 0.001}$), while a strong positive correlation was observed with RS (Figure 6b; $\boldsymbol{p < 0.001}$). Conversely, in Gymnosperms, α-diversity showed non-significant correlations with both SLA and RS (Figure 6d–f; $\boldsymbol{p > 0.05}$), but these relationships were not statistically significant ($\boldsymbol{p > 0.05}$). These results highlight the contrasting roles of leaf structural traits in shaping fungal diversity between the two evolutionary lineages, with Angiosperms displaying stronger trait-driven patterns than Gymnosperms.

4 | Discussion

4.1 | The Patterns of Phyllosphere Fungal α- and β-Diversity and Leaf Functional Trait Along the Elevation Gradient

This study investigated how phyllosphere fungal communities in Angiosperm and Gymnosperm host plants respond to variations in leaf traits and environmental factors along an elevational gradient, with a particular focus on their α-diversity. In Gymnosperms, α-diversity exhibited a monotonic decline with increasing elevation, consistent with previous studies on phyllosphere microbes (Wang et al. 2023), soil bacteria (Shigyo et al. 2019), and soil diazotrophs (Wang et al. 2019). This consistent decrease likely reflects elevational shifts in key influencing factors. Notably, both SLA and RS also declined monotonically with elevation (Figure 3a–c), mirroring the α-diversity trend observed in Gymnosperms. Moreover, RF importance ranking analysis identified RS as the most significant predictor of fungal α-diversity in Gymnosperms ($\boldsymbol{R^2 = 9.22\%; p < 0.05}$). Previous studies have shown that the functional traits of leaves, such as surface RS, can create microhabitats that influence microbial colonization (Liu et al. 2023). Rougher surfaces may provide more niches and protection for microorganisms, potentially promoting microbial adhesion, diversity, and resistance to environmental stressors (Leveau 2019; Mahnert et al. 2018).

Thus, the decline in phyllosphere fungal α-diversity in Gymnosperms along with increasing elevation likely results from a combination of leaf trait dynamics and environmental factors. Figure 4a,d highlight significant correlations between Gymnosperm fungal α-diversity and both AT and elevation, with a negative correlation coefficient ($R = -0.35$). Additionally, AT significantly decreased with increasing elevation (Supporting Information S1: Figure S1c), reinforcing the role of environmental

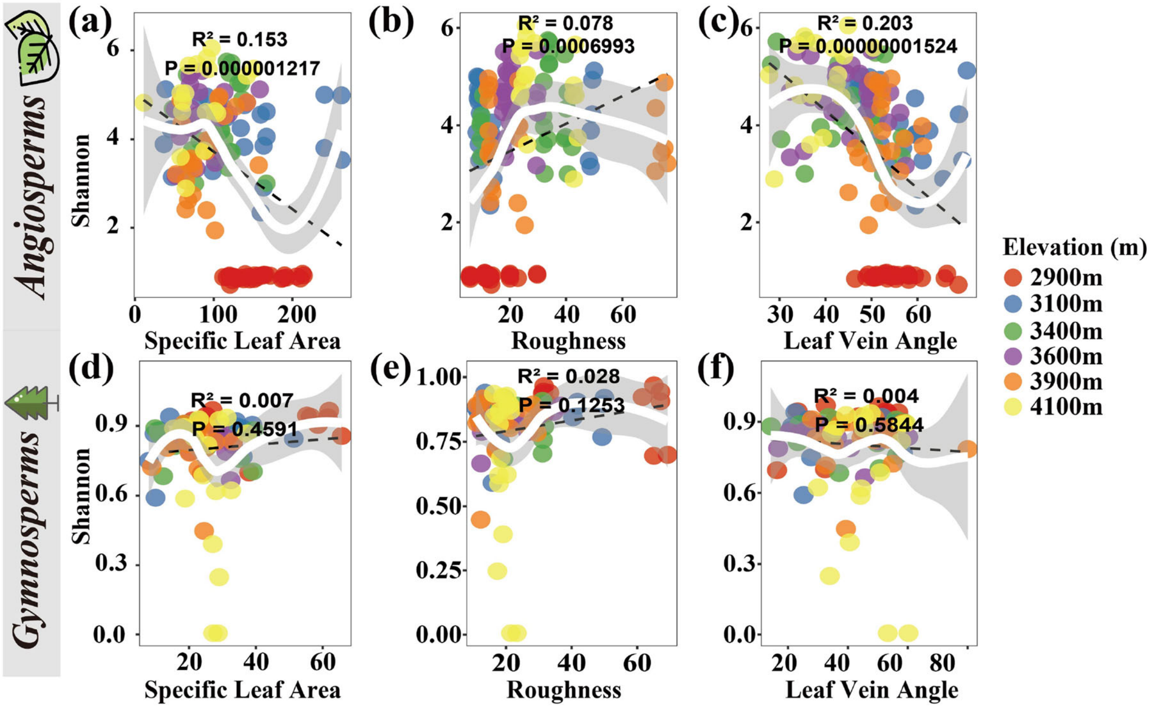

FIGURE 6 | Regression curves showing the relationship between fungal α-diversity and leaf functional traits (specific leaf area, roughness, and leaf vein angle) in Angiosperms (a–c) and Gymnosperms (d–f) across different elevations. A linear regression model with a two-sided test was used for statistical analysis, with adjusted R-squared values reported.

stressors in shaping fungal diversity. Lower temperatures at higher elevations constrain metabolic activity and fungal growth, limiting colonization and establishment on Gymnosperm leaves (Siles and Margesin 2016; Bahram et al. 2018). These temperature reductions also slow nutrient cycling and organic matter decomposition, restricting fungal resource availability and proliferation (Bahram et al. 2018). Moreover, colder conditions are associated with lower evapotranspiration rates, which reduce moisture availability on leaf surfaces and further limit microbial colonization and diversity (Vorholt 2012; Vacher et al. 2016). Consequently, environmental filtering intensifies at higher elevations, amplifying deterministic community assembly in Gymnosperms, where both leaf traits and environmental conditions jointly influence microbial assembly dynamics (Figure 4a,d).

The distinct elevational patterns observed between the Angiosperm and Gymnosperm fungal communities suggest fundamentally different community assembly mechanisms and niche filtering processes for these two evolutionary lineages. Whereas Gymnosperms exhibited a monotonic decline in α-diversity with increasing elevation, Angiosperms displayed a cosine-shaped pattern (**Slope = 0.002**, Figure 2), similar to trends reported for ammonia-oxidizing bacteria (Kou et al. 2023), soil bacteria (Peay et al. 2017), and soil methanotrophs (Li et al. 2021). These contrasting patterns reflect distinct ecological strategies: elevation emerged as the primary factor shaping fungal communities in Gymnosperms, whereas multiple leaf traits—including SLA, RS, and LVA—were dominant drivers of α-diversity in Angiosperms. Unlike prior studies of phyllosphere fungal diversity that reported reductions in canopy height in tropical forests (Izuno et al. 2016; Wang et al. 2023), the observed cosine trend in Angiosperms underscores their adaptive flexibility and complex trait-mediated responses to elevational gradients, which promote greater niche diversity and resilience to environmental fluctuations.

Previous studies (Yan et al. 2022) have suggested that fungal communities are influenced by the microhabitats formed by leaf surface traits. Rougher leaf surfaces create microsites that support microbial colonization by offering structural complexity, shelter from environmental stressors, and localized resource enrichment, thereby promoting higher fungal diversity and functional stability (Mahnert et al. 2018). For example, a study on cranberry (Vaccinium macrocarpon) leaves found significant differences in fungal colonization between older, rougher leaves and younger, smoother leaves (Mechaber et al. 1996). The rougher surfaces were associated with a higher diversity of fungal communities due to the increased availability of protective niches, moisture retention zones, and nutrient-rich microenvironments that support microbial colonization and metabolic activity (Mechaber et al. 1996), thereby supporting higher microbial diversity.

Moreover, venation traits such as LVA also influence phyllosphere microbial communities by affecting moisture retention, gas exchange, and nutrient distribution (Couturier et al. 2009; Sack and Scoffoni 2013). A larger LVA facilitates nutrient and water transport, creating spatial heterogeneity that supports niche differentiation and fungal aggregation, thereby improving photosynthesis efficiency (Cohu et al. 2014). Additionally, leaf angles also affect plant gas exchange capacity, contributing to more efficient photosynthesis and respiration (Zhou et al. 2020). Conversely, smaller LVAs—often associated with Angiosperms at higher elevations—form compact vascular networks that enhance microbial adhesion and stability under environmental stressors, contributing to greater fungal diversity and resilience under fluctuating environmental conditions (Cohu et al. 2014; McElwain et al. 2016).

These structural adaptations emphasize the importance of leaf venation architecture in regulating fungal diversity, community assembly, and functional stability along environmental gradients. With elevational increases, air humidity rises and AT declines significantly (Supporting Information S1: Figure S6). At higher elevations, Angiosperms exhibit reduced SLA and smaller LVAs (Figure 3a; $p < 0.05$), which limit microbial habitat availability. However, compact venation patterns promote microbial adhesion and stability, buffering environmental fluctuations and sustaining microbial diversity along elevational gradients (Cohu et al. 2014; McElwain et al. 2016). LVA also gradually decreases with elevation, especially at high elevations, due to suppressed plant growth rate and inhibited cell division and expansion under low-temperature conditions (Ma et al. 2021). The growth direction and shape of the leaves also change, resulting in a decrease in the relative angle between the main vein and the lateral vein (Nilsen et al. 2014).

The cosine trend may also be attributed to interactions between environmental gradients and plant physiological adjustments. Specifically, the mean canopy stomatal conductance and its sensitivity to vapor pressure deficit (VPD) increase with elevation in Angiosperms, such as *Schima superba* and *Castanopsis chinensis*, while Gymnosperms, like *Pinus massoniana*, maintain a relatively constant VPD sensitivity across elevations (Zhu et al. 2023). This differential response reflects contrasting hydraulic strategies, where Angiosperms adopt stricter stomatal regulation to optimize water-use efficiency, while Gymnosperms exhibit more anisohydric behavior, tolerating greater fluctuations in water potential (Zhu et al. 2023). In cold environments, plants need to manage water more effectively (Spooren et al. 2024). At lower elevations, higher temperatures and light availability promote rapid growth, resulting in higher SLA and more open vein structures, creating favorable conditions for fungal colonization. As elevation increases, temperature decreases significantly (Supporting Information S1: Figure S1), leading to smaller SLA and reduced LVA (Figure 3a; $p < 0.05$), which limit microbial habitat availability and resource diffusion. Reducing the angle between the main vein and the lateral vein can improve the water retention capacity of the leaves and reduce the surface area of water evaporation, thus helping plants survive under water-deficient conditions (Duncan 1971; Koike and Hotta 1996).

Phyllosphere fungi employ adaptive strategies to survive in harsh, high-elevation environments characterized by cold temperatures, low oxygen, and rapid climatic shifts (Zhu et al. 2022; Sohrabi et al. 2023). These strategies, including biosurfactant production, improved water retention (Wu et al. 2021), and stomatal regulation, enhance plant resilience and highlight the ecological diversity of microorganisms (Faticov et al. 2021). By stabilizing ecosystems and supporting plant adaptation and recovery, these microbial communities provide critical mechanisms for mitigating climate change impacts. Conserving functionally diverse microbial taxa and integrating microbial traits into biodiversity conservation frameworks are essential steps for maintaining ecological resilience in rapidly changing environments.

Analysis of β-diversity further underscored the contrasting ecological strategies of Angiosperms and Gymnosperms. Our β-diversity analysis revealed distinct elevation-driven patterns in the phyllosphere fungal communities of both groups, demonstrating how environmental filtering and plant functional traits selectively shape community composition, species turnover, and ecological assembly processes. In Angiosperms, fungal communities exhibited pronounced differentiation between low (2900 m) and mid-to-high elevations (3600–4100 m), driven by selective pressures associated with SLA, RS, and complex LVA. These traits enhance niche heterogeneity and reduce compositional homogenization (McElwain et al. 2016; Zhou et al. 2020).

In contrast, Gymnosperms showed greater structural overlap in fungal community structure at low-to-mid elevations (2900–3600 m), with significant divergence only at higher elevations. This pattern reflects their lower SLA, smoother leaf surfaces, and simpler vein structures, which constrain niche availability and promote deterministic assembly processes (Brodribb and Feild 2010; Sack and Scoffoni 2013). Species turnover, rather than nestedness, primarily drives β-diversity in Gymnosperms *(*da Silva et al. 2018*)*, highlighting the influence of environmental filtering at higher elevations (Zhou and Ning 2017).

Angiosperms maintained greater community differentiation across elevations, driven by functional traits that support adaptive flexibility and reduce compositional homogenization under environmental stress. These functional adaptations enabled Angiosperms to maintain higher fungal abundance and structural stability along the gradient, underscoring the contrasting assembly dynamics and ecological strategies of the two evolutionary lineages in shaping phyllosphere fungal diversity across elevational gradients.

4.2 | The Phyllosphere Fungal Community Composition and Co-Occurrence Networks Along the Elevation Gradient

Across all elevations, *Ascomycota* dominated the phyllosphere fungal communities in both Angiosperms and Gymnosperms, with its relative abundance peaking at 4100 m. However, Angiosperms exhibited more stable relative abundances of fungi phyla—including *Ascomycota*, *Basidiomycota*, and *Chytridiomycota*—across mid-elevations (3100–3900 m), whereas Gymnosperms exhibited greater variability and stronger shifts in composition. Leaf-surface water droplets also affect nutrient distribution (van der Wal and Leveau 2011; Beattie 2011). Collectively, *Ascomycota*, *Basidiomycota*, and *Chytridiomycota* accounted for over 75% of the total fungal communities across all elevations, highlighting their ecological significance in maintaining ecosystem function (Perreault and Laforest-Lapointe 2022). *Ascomycota* drives organic matter decomposition and nutrient cycling, *Basidiomycota* contributes to pathogen defense and lignin degradation, and *Chytridiomycota* supports moisture-dependent recovery processes (Sohrabi et al. 2023). These functions enhance plant colonization, ecological restoration, and long-term stability, especially in response to climate variability (Zhu et al. 2022). Conserving these fungi and their functional traits is thus essential for sustaining biodiversity amid global environmental change.

The relative stability of fungal community composition in Angiosperms is likely driven by leaf traits such as higher SLA, greater RS, and larger LVA, which create diverse microhabitats, enhance nutrient retention, and buffer environmental changes along elevational gradients (Yan et al. 2022). Consistent with studies on bacterial communities, microtopographic features—such as vein density and RS—are critical in shaping microbial stability by providing ecological niches and promoting resource retention (Lindow and Brandl 2003; Yan et al. 2022). Higher vein density in Angiosperm leaves likely provides a greater number of ecological niches, supporting fungal diversity and stability. In contrast, the lower vein density and simpler leaf structures of Gymnosperms may limit niche availability, resulting in greater variability in fungal community composition across elevations. Their smoother surfaces, lower SLA, and simpler vein structures further constrain microbial colonization, generally supporting less diverse and more variable fungal α-diversity (Mechaber et al. 1996). These patterns suggest that Angiosperms, due to their complex leaf traits, foster more stochastic community assembly, contributing to stable diversity across elevations. Conversely, Gymnosperms are shaped by deterministic processes, reinforcing the influence of leaf microtopography on fungal diversity patterns along environmental gradients (Yan et al. 2022).

The co-occurrence networks of phyllosphere fungi revealed distinct elevation-driven adaptations between Angiosperms and Gymnosperms. In Angiosperms, network average path length initially decreased with elevation but increased significantly at mid-elevations (3400–3600 m), suggesting dynamic adjustments to environmental stressors. This pattern corresponded with a non-linear shift in the ratio of positive to negative interactions, which declined at 3100–3400 m before increasing again at higher elevations. Co-occurrence network analysis showed that the ratio of positive to negative microbial interactions varied in parallel with the patterns of fungal diversity and leaf traits along the elevational gradient in Angiosperms. Such positive interactions enhanced network connectivity and promoted stability, highlighting the capacity of Angiosperm fungal networks to withstand environmental fluctuations. At high elevations, enhanced positive interactions among Angiosperms improve network connectivity, strengthening ecosystem stability and resilience. Mutualistic relationships, such as nitrogen-fixing bacteria aiding nutrient uptake and defense activation, play a role in mitigating environmental stress.

In contrast, Gymnosperms show higher proportions of negative interactions, leading to weaker network connectivity and reduced resilience. This contrast highlights evolutionary differences in plant-microbe interactions and emphasizes the importance of managing mutualisms to buffer against environmental pressures. Future research should leverage metagenomics and network analyses to explore microbial dynamics across spatial and temporal scales, advancing strategies for biodiversity conservation and ecosystem resilience in high-altitude environments.

These adaptations in Angiosperms are likely facilitated by higher SLA, greater RS, and more complex LVA, which collectively provide diverse niches, improve nutrient retention, and buffer stressors (Zhou et al. 2020; Yan et al. 2022). In contrast, Gymnosperms exhibited a steady increase in network complexity at high elevations, reflected by a greater number of nodes and edges. However, the positive-to-negative interaction ratio consistently declined, leading to weaker connectivity despite increased structural complexity. The smoother surfaces, lower SLA, and simpler vein structures in Gymnosperm leaves constrain niche availability, limiting microbial colonization and promoting deterministic processes that reduce the adaptive flexibility of their fungal networks under environmental stress (Brodribb and Feild 2010).

Collectively, these findings demonstrate how differences in SLA, RS, and LVA between evolutionary lineages influence fungal community assembly, network resilience, and ecological responses to environmental gradients.

4.3 | The Assembly Process of the Phyllosphere Fungal Community Along the Elevation Gradient

The assembly processes of phyllosphere fungi in Angiosperms and Gymnosperms along the elevation gradient exhibit distinct dynamics, influenced by evolutionary history and leaf microtopographic traits. Our findings reveal that deterministic processes predominantly governed the phyllosphere fungal community assembly across all elevations, with their contribution intensifying at higher elevations, supporting the environmental filtering hypothesis (Zhou and Ning 2017). However, Angiosperms exhibited a greater degree of stochasticity than Gymnosperms, as indicated by higher MST values across most elevations. This increased stochasticity underscores the adaptive flexibility of Angiosperms, which is likely facilitated by their higher SLA, greater RS, and more complex LVA—traits that provide diverse microhabitats, enhance microbial adhesion, and buffer environmental stress (McElwain et al. 2016; Zhou et al. 2020).

In contrast, Gymnosperm communities were predominantly shaped by deterministic processes, with stochastic contributions consistently declining along the elevation gradient. The simpler leaf structures of Gymnosperms—characterized by lower SLA, smoother surfaces, and less complex venation patterns—limit niche availability and amplify selective pressures, leading to homogeneous community assembly dominated by deterministic processes (Brodribb and Feild 2010; Sack and Scoffoni 2013). This deterministic dominance reflects the evolutionary constraints of Gymnosperms, which emphasize resource conservation and stress tolerance, particularly at higher elevations.

The interaction between evolutionary history and elevation (Taxa × Elevation) significantly influenced MST values, underscoring distinct assembly dynamics between these two evolutionary lineages. As an ancient clade that diverged over 300 million years ago, Gymnosperms evolved conservative traits—including low SLA, smooth surfaces, and simpler venation patterns—adapted for resource conservation and environmental stress tolerance (De La Torre et al. 2017). These traits restrict microbial colonization, resulting in lower α-diversity and deterministic assembly processes at higher elevations (Sack and Scoffoni 2013). In contrast, Angiosperms, which evolved later, developed structurally complex leaves with higher SLA, greater

RS, and larger LVA, enhancing resource acquisition and promoting ecological plasticity (Díaz et al. 2016). These adaptive traits provide diverse microhabitats, promote microbial adhesion, and buffer against environmental fluctuations, enabling higher α-diversity and more stochastic assembly processes, even under decreasing temperatures (Zhou et al. 2020; Yan et al. 2022).

Such evolutionary innovations demonstrate how Angiosperm leaf traits drive microbial diversity and resilience, whereas Gymnosperms rely on deterministic strategies shaped by environmental filtering. Collectively, these findings emphasize the synergistic effects of evolutionary history, leaf functional traits, and environmental factors—such as temperature and elevation—in shaping the contrasting assembly mechanisms and divergent patterns of fungal community structure between Angiosperms and Gymnosperms. While deterministic processes ensured community stability in Gymnosperms, Angiosperms maintained higher α-diversity and greater resilience to elevational gradients, further highlighting the role of microtopographic traits in promoting stochastic assembly and ecological adaptability along environmental gradients.

High-elevation regions, vulnerable to climate change and environmental stressors, require prioritized biodiversity conservation efforts, while mid-elevation zones—identified as Angiosperm diversity hotspots— demand targeted restoration strategies to sustain ecological stability (Zhu et al. 2023). Angiosperm traits, such as LVAs and RS, influence microbial diversity and community assembly, thereby enhancing habitat adaptability and ecosystem resilience (McElwain et al. 2016). Conversely, Gymnosperms, characterized by conservative traits, support lower microbial diversity and therefore require protection in low-elevation habitats to maintain nutrient cycling and stress tolerance (Pandey et al. 2021; Zhu et al. 2023). Consequently, integrating plant functional traits and microbial networks into conservation frameworks offers adaptive strategies to strengthen ecosystem resilience and mitigate the impacts of environmental change.

5 | Conclusion

This study demonstrates that evolutionary history and leaf functional traits are fundamental in shaping phyllosphere fungal communities along a subalpine elevational gradient. We identified significant differences in α-diversity, community composition, and co-occurrence network interactions between Angiosperms and Gymnosperms. SLA, RS, and LVA emerged as key drivers of fungal diversity, with Angiosperms exhibiting stronger associations with these traits than Gymnosperms. At higher elevations, deterministic processes predominantly structured fungal communities, while stochastic processes played a greater role at mid-elevations, particularly in Angiosperms. These findings underscore the importance of evolutionary history and leaf traits in shaping fungal diversity patterns and community assembly processes across elevational gradients.

Furthermore, fungi demonstrate adaptive strategies to environmental gradients, playing a vital role in plant-microbe interactions and ecosystem resilience. Building on these insights, conservation strategies should prioritize the protection of core fungal taxa, the safeguarding of the few Angiosperm species at higher elevations, and the preservation of fragile alpine and subalpine ecosystems. Strengthening plant-microbe interactions and incorporating leaf trait-based models are essential for improving ecosystem resilience and enabling leaf habitats to adapt to environmental shifts, thereby supporting long-term biodiversity conservation.

Author Contributions

Xiaocheng Yu: investigation, writing – review and editing, writing – original draft, visualization, validation, methodology, data curation, formal analysis, software. **Yuxuan Mo:** methodology, writing – original draft, writing – review and editing, visualization, validation, software. **Yuehua Hu:** writing – original draft, writing – review and editing, methodology, validation. **Zhaoqiao Wu:** methodology, writing – original draft, visualization, writing – review and editing, software. **Qiang Luo:** writing – original draft, writing – review and editing, methodology. **Liang Song:** methodology, writing – original draft, writing – review and editing. **Zhenghong Tan:** writing – review and editing, writing – original draft, methodology. **Hua-Zheng Lu:** conceptualization, methodology, writing – original draft, writing – review and editing, visualization, project administration, funding acquisition, resources, supervision, investigation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

We recognize the importance of data sharing in academic research and fully support this practice. We are willing to provide access to the data for fellow researchers as needed. Researchers interested in obtaining the data can contact the authors via email. The data that supports the findings of this study are available in the Supporting Information of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Figure S1: Variation of environmental parameters along an elevation gradient (2900, 3100, 3400, 3600, 3900, and 4100 m) within the microhabitats where host plants were sampled. **Figure S2:** The β-diversity patterns fungal communities in angiosperms and gymnosperms. **Figure S3:** The Rarefaction curves of phyllosphere fungal communities along with elevations (2900, 3100, 3400, 3600, 3900, and 4100 m) in angiosperms (a) and gymnosperms (b). **Figure S4:** The community composition of fungal communities in angiosperms and gymnosperms. **Figure S5:** The community assembly of fungal communities in angiosperms and gymnosperms. **Table S1:** A list of angiosperm and gymnosperm species sampled along an elevational gradient is provided. **Table S2:** Similarity values among samples across the 2900, 3100, 3400, 3600, 3900, and 4100 m elevations, were analyzed using PERMANOVA (Permutational Multivariate Analysis of Variance) with pairwise ANOSIM tests. The statistical results are presented in the table. **Table S3:** Effects of host plant taxa (T), elevation (E), and interaction (T × E) on the fungal community assembly, analyzed through generalized linear mixed models. **Table S4:** Co-occurrence networks of phyllosphere fungal communities in angiosperms and gymnosperms. Topological characterizations are presented along the elevation gradient (2900, 3100, 3400, 3600, 3900, and 4100 m).