


Linking root-associated fungal and bacterial functions to root economics

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Abstract

Tree roots form symbioses with soil microbes to acquire nutrients, but the relationships between root nutrient acquisition strategies and microbial community composition remain poorly understood. Here, we measured root traits and root-associated fungal and bacterial guilds in 336 trees of 52 species from a subtropical forest. We found a fungal gradient from ectomycorrhizal to saprotrophic dominance, which corresponded with a shift from organic to mineral nutrient economics. This fungal gradient was aligned with the increase of root nitrogen concentration, suggesting a linkage from simple root trait to fungal-mediated carbon-nutrient cycling. We also found that the functional composition of fungal and bacterial communities was closely correlated with host root-zone pH, which often varied among coexisting trees. Root-zone pH was independent of the common root traits, underpinning a potential new gradient in the root trait space. Our findings integrate microbial functions into the root economics framework, thereby advancing the understanding of diversity of nutrient acquisition strategies across forest trees.

eLife assessment

This **valuable** study advances our understanding of the below-ground resource acquisition strategies of diverse tree species, integrating the roles of both roots and their associated microbes. The support for the conclusions is **incomplete** owing to the uncertainties or shortcomings associated with the design and statistical analyses. Regardless of these technical issues, this study can be of broad interest for plant and microbial ecologists.

Introduction

Plant species have evolved a diversity of root forms and functions to facilitate resource uptake from a wide range of environments (1, 2). This belowground root diversity plays a critical role in regulating the structure and functioning of terrestrial ecosystems (3).

Species with different root traits are suggested to vary along a fast-slow economics spectrum, ranging from inexpensive, short-lived roots with high metabolic activity to expensive, long-lived roots with lower metabolic activity (4–7). However, roots do not act alone; they associate with soil fungi and bacteria with various functions to enhance nutrient acquisition. Thus, incorporating the functions of root-associated fungal and bacterial communities into the root economics framework may better capture the holistic diversity of belowground strategies in plant nutrient acquisition.

In particular, most plants form associations with mycorrhizal fungi to increase their access to soil resources. Linking the functions of mycorrhizal fungi with the root economics framework results in a two-dimensional trait space (8). In this root economics space, one axis represents how quickly resources are invested and returned, and the other axis describes where the nutrient resources are mainly captured, either roots or root-associated mycorrhizal fungi. The mycorrhizal dependence is then strongly and positively correlated with root diameter, explaining most of the variation in the root economics of nutrient acquisition (8).

However, this collaboration framework still requires refinement. For example, the assumed positive relationship between root diameter and mycorrhizal dependence may only occur for arbuscular mycorrhizal (AM) fungi. Thicker absorptive roots usually have a larger cortical volume (9), which permits greater colonization by AM fungi (10). Cortical volume, however, may not directly influence the colonization and nutrient uptake by ectomycorrhizal (EcM) fungi because the EcM structures are often limited to the outer layers of the roots (11,12). Additionally, AM fungi primarily absorb nutrients mineralized by saprotrophic microorganisms (13,14), whereas many EcM fungi are able to bypass the mineralization pathway and directly mine nutrients from organic sources (15). Clearly, it is important to consider the differences between AM and EcM fungi with respect to the root-fungal collaboration gradient.

A second limitation of the collaboration framework is its explicit focus on the complementary role of mycorrhizal fungi in root nutrient acquisition without considering other ecologically relevant fungal and bacterial taxa (3). Roots collaborate with a variety of fungal and bacterial taxa to facilitate nutrient uptake (16). The relative abundance of fungal and bacterial guilds across plant hosts may mediate the plant's strategy in nutrient acquisition. For example, a higher relative abundance of nitrogen-fixing bacteria may indicate a lower dependence on soil nutrients (17). In addition, a higher relative abundance of saprotrophic fungi can enhance the mineralization of rhizosphere organic nutrients (18–21), potentially promoting a mineral nutrient economy surrounding the roots (22).

Despite the critical role of microbial communities in mediating nutrient acquisition strategies, microbial functions have been largely overlooked in trait-based economics frameworks of plant roots. However, increasing evidence suggests that root-associated microbial community composition can be partly explained by root traits (23–28). On the other hand, roots interact with soil in ways that also shape microbial communities (12, 28–30). Therefore, disentangling the roles of root traits and soil properties in influencing the relative abundance of root-associated fungal and bacterial guilds may make it possible to explicitly incorporate microbial functions into the root economics framework of nutrient uptake.

Here, we surveyed the functional guilds of fungal and bacterial communities in the rhizosphere (root adherent soil) and within the absorptive roots (1st–3rd orders, 31) across 336 tree hosts of 52 species from a subtropical forest (Table S1). These trees were distributed along a nearly 1000-m elevational gradient and formed either AM or EcM symbioses. Our goal was to integrate the functions of root-associated microbial communities into the existing root economics space across a broad range of plant species.

Results

The root-associated fungal and bacterial communities

We identified 2,037 fungal and 2,348 bacterial ASVs in the rhizosphere soil, with similar ASV richness in the root tissue. A higher proportion of fungal sequences (76.7% in rhizosphere, 65.2% in roots) were annotated with a functional guild than bacterial sequences (33.2% in rhizosphere, 45.9% in roots) (**Fig. 1**, **S1**; **Table S2**). In both rhizosphere soil and root tissue, saprotrophic fungi were the most abundant fungal guild (mean 42.9% in rhizosphere, 30.2% in roots), followed by ectomycorrhizal (EcM) fungi (19.0% in rhizosphere, 15.9% in roots). Plant pathogenic fungi and AM fungi were relatively less abundant (**Fig. 1**, **S1**; **Table S2**). AM fungi accounted for only 0.7% of the rhizosphere ITS reads, while their relative abundance based on qPCR was 1.6% (**Table S2**). In the bacterial communities, taxa with the function of nitrogen fixation (5.1% in rhizosphere, 7.9% in roots) and nitrogen reduction (3.2% in rhizosphere, 3.1% in roots) were the dominant nitrogen-related guilds, while the most abundant carbon-related guild was the functional group of other aerobic chemoheterotrophs (**Fig. 1**, **S1**; **Table S2**).

Factors influencing the compositions of fungal and bacterial guilds

Marginal tests of the dbRDA revealed that the compositions of fungal guilds in both the rhizosphere and root tissue were mainly explained by host mycorrhizal type (9.6% in rhizosphere, 13.8% in roots), elevation (2.9% in rhizosphere, 0.9% in roots), root [N] (3.0% in rhizosphere, 0.8% in roots), root-zone soil pH (5.6% in rhizosphere, 2.6% in roots) and soil gravimetric water content (1.9% in rhizosphere, 0.8% in roots) ($P \leq 0.05$, **Tables 1**, **S3**). In contrast, the composition of bacterial guilds in both the rhizosphere and root tissue was largely unrelated to mycorrhizal type and absorptive root traits, but was strongly explained by root-zone soil pH (21.3% in rhizosphere, 13.4% in roots, **Tables 1**, **S3**).

In the root economics space incorporating the guilds of fungal communities, one gradient mainly reflected the variation in root diameter, specific root length, and root tissue density. Another gradient represented all fungal guilds and root [N] (**Fig. 2**, **Tables S4-S6**). In general, trees with higher root [N] tended to be associated with a greater relative abundance of saprotrophic fungi, plant pathogenic fungi, and AM fungi, but a lesser relative abundance of EcM fungi than trees with lower root [N]. This was true regardless of the sampling compartment (rhizosphere soil vs. root tissue), analysis level (individual vs. species level), or estimation method (sequencing-based or qPCR-based relative abundance of AM fungi) (**Fig. S2**, **Tables S4-S6**).

In the root economics space incorporating the guilds of bacterial communities, the gradient of bacterial guilds was strongly associated with root-zone soil pH (**Figs. 3**, **S3**). Along the root-zone soil pH gradient ranging from approximately 3.5 to 7.5 across the host individuals (or from 4.0 to 6.4 at the species level), the relative abundance of rhizosphere bacterial taxa possessing the fermentation function decreased, while the relative abundance of other bacterial guilds increased (**Fig. 4**). The remaining gradients mainly represented other root-zone soil properties, as well as host root morphology and tissue density (**Tables S7-S8**).

Discussion

The gradient of fungal guilds

When we incorporated fungal functions into the root economics space, we identified a gradient spanning from EcM to saprotrophic fungal dominance in both rhizosphere soil and root tissue of 336 subtropical trees (**Fig. 2**). This gradient of fungal guilds was also observed in the species-level PCAs (**Fig. S2**). Many EcM fungal taxa can obtain nutrients directly from organic sources

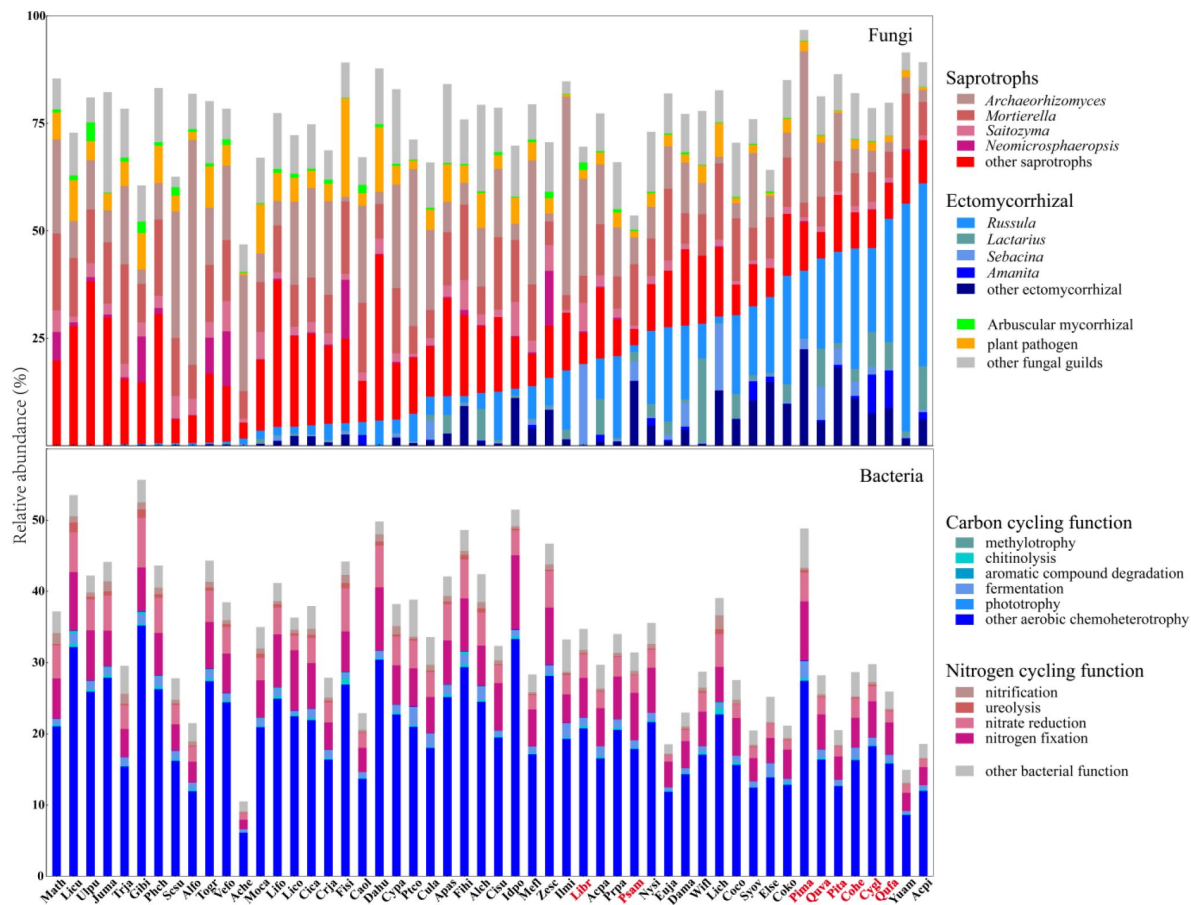


Fig. 1.

Compositions of fungal and bacterial guilds in the rhizosphere soil across 52 tree species.

The relative abundance of fungal (upper panel) and bacterial (lower panel) guilds is estimated based on sequencing reads. Compositions of the five most abundant ectomycorrhizal (EcM) and saprotrophic genera are also shown. Abbreviations of EcM tree species are in red font, while arbuscular mycorrhizal (AM) tree species are in black font. Complete scientific names of trees are provided in [Table S1](#).

	Fungi			Bacteria		
	var%	F	<i>P</i>	var%	F	<i>P</i>
Elevation	2.9%	11.6	0.001	4.5%	19.4	0.001
Mycorrhizal type	9.6%	37.9	0.001	0.7%	2.8	0.053
Root diameter	0.2%	0.7	0.51	0.3%	1.2	0.30
Specific root length	0.1%	0.4	0.72	0.2%	1.1	0.36
Root [N]	3.0%	11.9	0.001	1.8%	7.8	0.002
Root tissue density	0%	0	1.0	0.4%	1.6	0.18
Root-zone soil pH	5.6%	22.2	0.001	21.3%	92.7	0.001
Soil water content	1.9%	7.3	0.003	0.3%	1.3	0.27
Soil total carbon	0%	0	1.0	0.6%	2.4	0.087
Soil total nitrogen	0.2%	0.8	0.43	0.4%	1.8	0.16
Full model	51.6%	32.3	0.001	45.2%	25.0	0.001

Table 1.

Distance-based redundancy analysis (dbRDA) to determine the predictor variables that significantly influenced the functional compositions of rhizosphere fungal and bacterial communities.

Predictor variables include elevation, host mycorrhizal type, absorptive root traits, and root-zone soil properties. Results show marginal tests based on the Manhattan distance matrix, where var% indicates the relative contributions of predictor variables to fungal and bacterial guild dissimilarity. The marginal test assess each marginal term analyzed in a model with all other variables.

The relative abundance of AM fungi is estimated by ITS sequencing in this table. Significant statistics ($P < 0.05$) are indicated in bold.

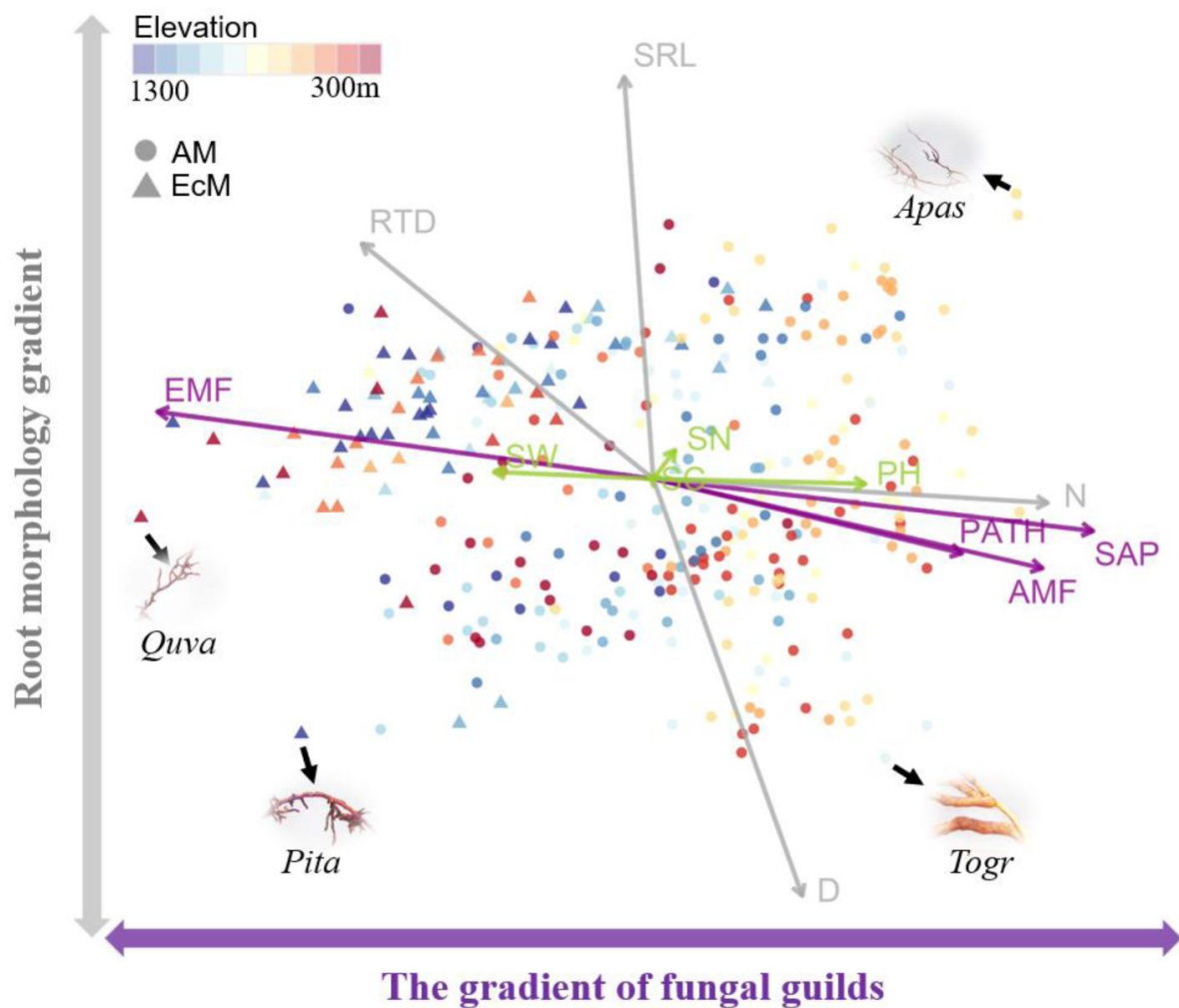


Fig. 2.

The gradient of rhizosphere fungal guilds in the root economics space.

Principal component analysis followed by varimax rotation was performed on root traits (grey), root-zone soil properties (green), and relative abundances of different fungal guilds (purple). In this trait space, there is a gradient ranging from ectomycorrhizal (EcM) to saprotrophic (SAP) fungal dominance. Greater relative abundance of plant pathogenic fungi (PATH), arbuscular mycorrhizal fungi (AMF, estimated by ITS sequencing here), and higher root nitrogen concentration (N) is associated with the dominance of SAP fungi. Other root traits, including root diameter (D), specific root length (SRL), and partly root tissue density (RTD), comprise the root morphology gradient. Root-zone soil properties, including root-zone pH (PH), gravimetric water content (SW), total carbon (SC), and nitrogen (SN) concentration, mainly occupy the gradient decoupled from most root traits and fungal guilds. Four representative tree species are highlighted with pictures of absorptive roots presented at the same scale: *Quercus variabilis* (Quva); *Pinus taiwanensis* (Pita); *Torreya grandis* (Togr); *Aphananthe aspera* (Apas).

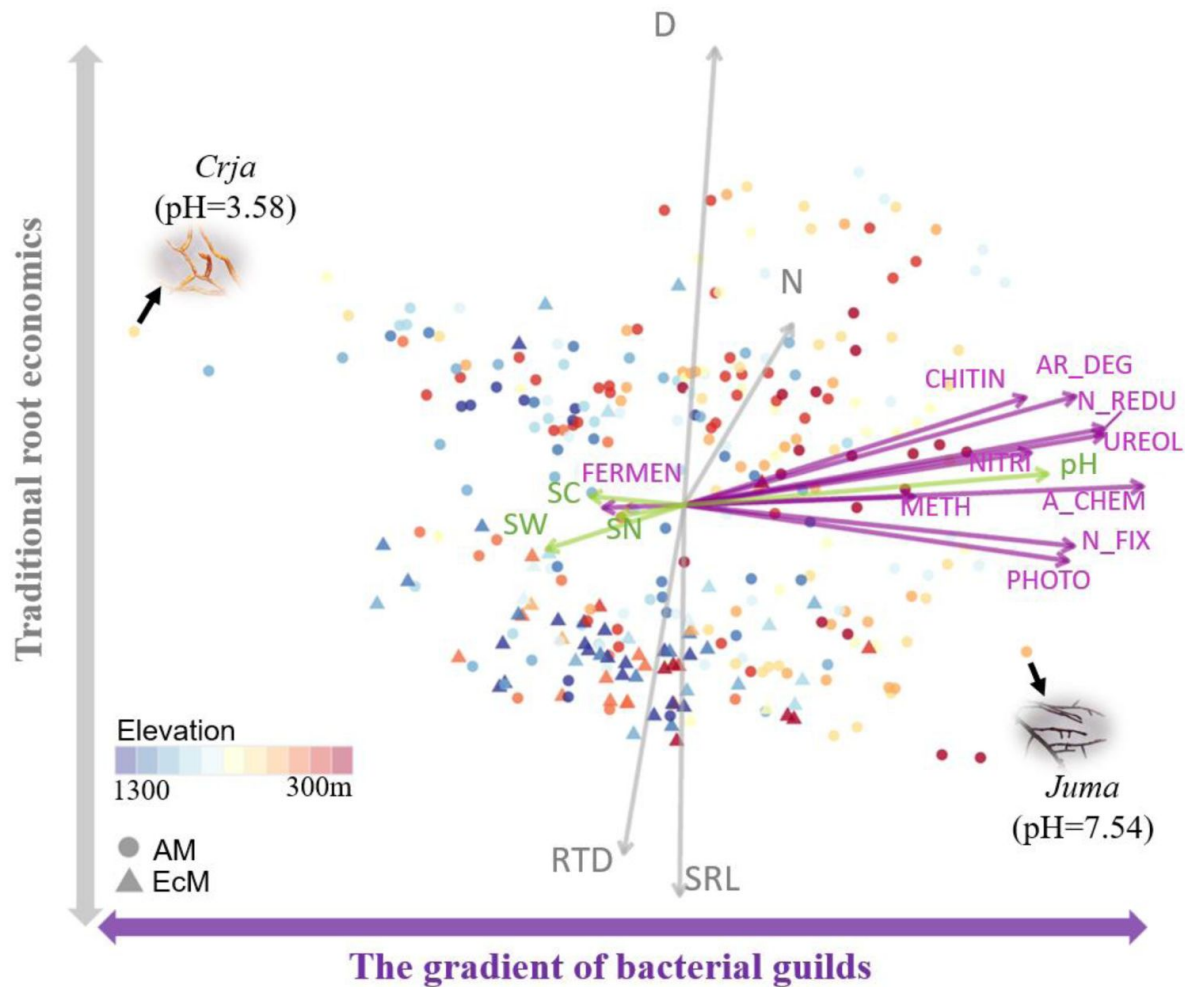


Fig. 3.

The gradient of rhizosphere bacterial guilds in the root economics space.

Principal component analysis followed by varimax rotation was performed on root traits (gray), root-zone soil properties (green), and relative abundances of different bacterial guilds (purple). The variation of most bacterial guilds, including aromatic compound degradation (AR_DEG), chitinolysis (CHITIN), ureolysis (UREOL), nitrogen fixation (N_FIX), nitrification (NITRI), nitrate reduction (N_REDU), methylotrophy (METH), phototrophy (PHOTO), and other aerobic chemoheterotrophy (A_CHEM), is strongly related to root-zone pH (pH), but not gravimetric water content (SW), total carbon (SC), and nitrogen (SN) concentration. The pH gradient is also largely decoupled from the traditional root economics traits, including root diameter (D), specific root length (SRL), root nitrogen concentration (N), and root tissue density (RTD). Two representative tree species are highlighted with pictures of absorptive roots presented at the same scale: *Cryptomeria japonica* var. *sinensis* (*Crja*) and *Juglans mandshurica* (*Juma*).

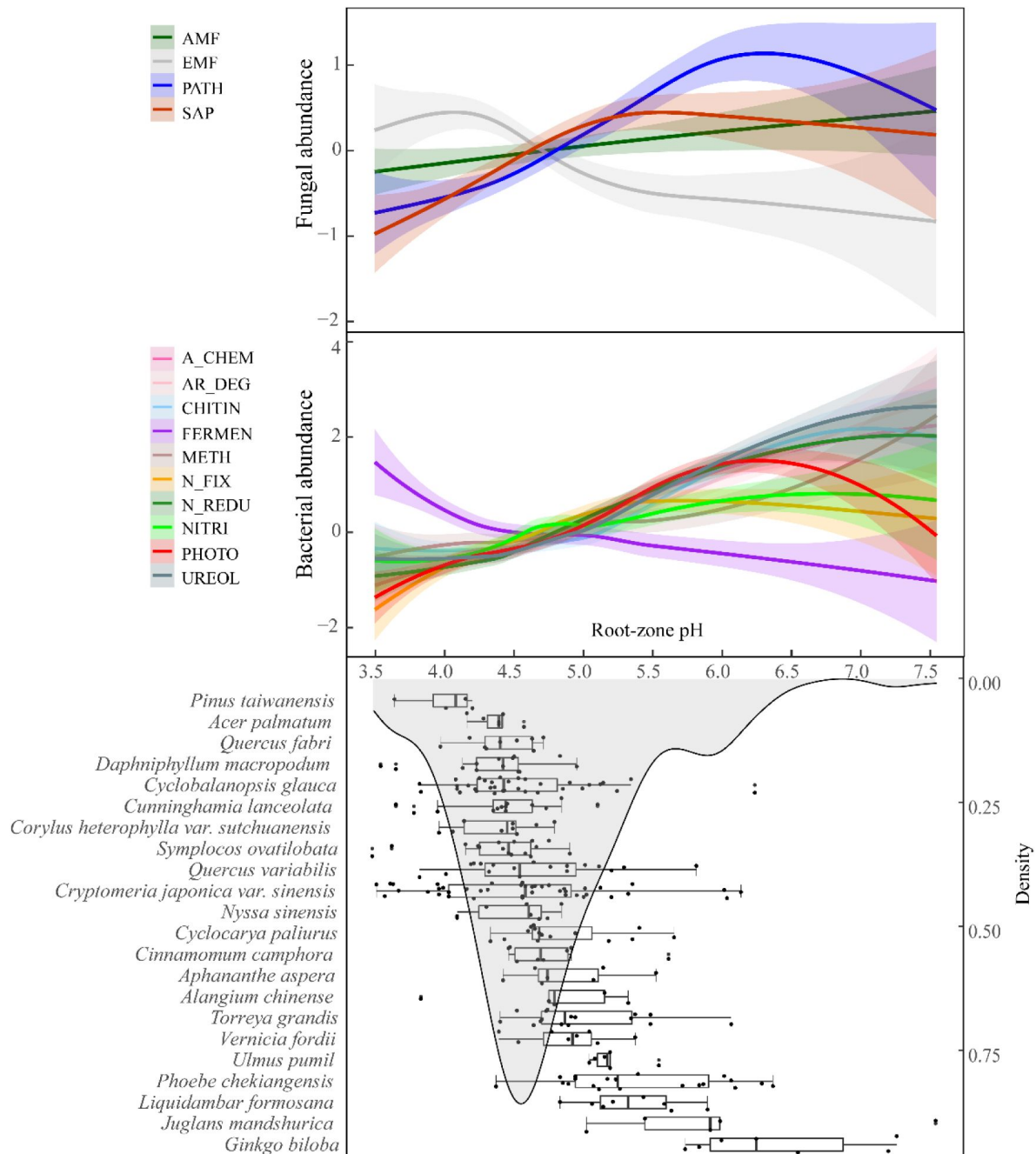


Fig. 4.

Shifts in rhizosphere fungal and bacterial functional compositions along the root-zone pH gradient.

The relative abundance of different fungal (top panel) and bacterial (middle panel) guilds in the rhizosphere is standardized, plotted along the pH gradient, and fitted with a smoothed curve. The relative abundance of arbuscular mycorrhizal fungi (AMF) is estimated by ITS sequencing in this figure. In the bottom panel, root-zone pH values of the common tree species in our dataset (sample size ≥ 4) are shown (box plots), with the shaded area indicating the density curve of pH across all 336 tree hosts. All three panels share the same pH scale.

(15), while saprotrophic fungi are responsible for nutrient mineralization from organic matter. Further, higher saprotrophic dominance was associated with higher proportions of AM fungi (Figs. 2, S2), which enhance mineral nutrient uptake. Thus, our data clearly showed a gradient from hosts associated with fungi capable of mining organic nutrients to hosts associated with fungi that more frequently use mineralized nutrients. This gradient of root-associated fungal guilds may couple with the gradual change from organic to mineral nutrient economics across forest trees (22).

Consistent with observations in other subtropical trees, host mycorrhizal type (AM vs. EcM) was a strong factor in shaping the composition of fungal communities (Table 1; 32-33). On average, EcM trees hosted higher proportions of EcM fungi (45.1% in rhizosphere, 45.6% in roots) than AM trees (10.9% in rhizosphere, 6.1% in roots; both $P < 0.001$ in Kruskal-Wallis rank tests). However, we detected high relative abundances of EcM fungal taxa, including *Russula* and *Lactarius* species, associated with some trees that are traditionally considered to be AM (34). Although these EcM fungi may not form typical EcM structures such as mantles or a Hartig net when associating with AM tree hosts (35), they were found in both rhizosphere soil and root tissue (Figs. 1, S1). In addition, associations between EcM fungi and conventional AM tree species have also been reported in other subtropical forests (36-37). Thus, mycorrhizal type was not the only predictor of mycorrhizal and non-mycorrhizal fungal guilds.

Indeed, root [N] explained significant variation of fungal functional composition. Higher root [N] often indicates rapid root respiration and exudation in woody species, denoting a fast root metabolic economy, whereas woody species with low root [N] typically have lower metabolic rates (6-8, 38). Thus, the trait-related root economics and fungal-driven nutrient economics may be integrated into a unified framework (Fig. 2). Moreover, our data suggest that in this integrated economics framework, the gradient of fungal guilds was largely decoupled from the axis of root morphology, suggesting an alternative gradient of fungal collaboration different from the traditional root morphology-driven framework predicting AM fungal colonization intensity (8).

This integrated economics framework largely explained the tree distribution patterns along the elevational gradient in our study site (Table 1). EcM fungi were more frequently dominant at higher elevations, where EcM trees, as well as AM trees with lower [N], were common. In contrast, saprotrophic fungi were more frequently dominant at lower elevations, where AM trees with higher [N] were more frequently distributed (Fig. 2). This distribution pattern was consistent with an increase in litter decomposition rate from high to low elevations (39, 40).

The integrated economics framework was also associated with variation in the relative abundance of plant pathogenic fungi (Figs. 2, S2, Tables 1, S3-S6). Colonization by EcM fungi reduces the possibility of infection by increasing pathogen resistance (41), which was supported by the negative relationships between EcM and plant pathogenic fungal abundances (Fig. 2). This negative relationship has been considered an important driver of conspecific seedling establishment within a local community dominated by EcM hosts (42, 43). Our results suggest that, in addition to mycorrhizal type, common root traits (e.g. root [N]) can also influence the abundance of EcM and plant pathogenic fungi, which may therefore explain the composition of tree species in forest communities.

The role of root-zone pH in the root economics space

The functional composition of bacterial communities in both rhizosphere soil and root tissue was often poorly explained by common root traits, but was strongly correlated to root-zone pH (Figs. 3-4, S3-S4, Tables 1, S7-S8). Along the pH gradient, low soil pH often decreases bacterial functional diversity (44) and may favor only certain bacterial guilds (e.g. fermentative bacteria in our system, Fig. S5). Also, low soil pH has been shown to inhibit chemoautotrophic

growth of ammonia oxidizers, suppressing nitrification and downstream nitrogen loss pathways (45 [↗](#)). However, the full ecological linkage between root-zone pH and bacterial functions requires further investigation.

Root-zone pH also influences the relative abundance of fungal guilds (Figs. 4 [↗](#), S4 [↗](#), Table 1 [↗](#)). EcM fungi are often associated with more acidic soils, probably because relatively lower pH is favored by EcM fungi or because EcM fungi actively lower the pH by producing organic acids to dissolve mineral forms of phosphorus and act as chelators that are important in the oxidation of soil organic matter (46 [↗](#)). As rhizosphere pH increases, AM fungi, saprotrophic fungi, and plant pathogenic fungi tended to become more abundant relative to EcM fungi (Fig. S4 [↗](#)). Thus, the composition of fungal guilds was partly associated with root [N] and partly associated with root-zone soil pH (Table 1 [↗](#)).

Importantly, root-zone soil pH was influenced by not only elevation, as previously reported (47 [↗](#)), but also tree host identity across the study landscape, or within a plot where the local pH range was not narrow (Site 1-S6, Table S9 [↗](#)). Therefore, in some areas, root-zone soil pH may be considered an intrinsic, measurable, tree species-specific trait. We suggest two potential causes of interspecific variation in root-zone pH. First, tree species may prefer different forms of mineral nitrogen (48 [↗](#)), with ammonium (NH_4^+) uptake being associated with the release of hydrogen ions (H^+) to the rhizosphere and lower pH, while nitrate (NO_3^-) uptake causes hydroxide (OH^-) release leading to increased pH (49 [↗](#)).

Second, tree species differ in their tissue calcium (Ca) concentration, and the Ca released by plant litter can mediate soil pH, as Ca^{2+} competes with H^+ and aluminum ions (Al^{3+}) for exchange sites on soil particle surfaces (50 [↗](#)). For example, *Pinus* species often exhibit lower pH in their rhizosphere than other species, probably because of the low Ca concentration in the tissue of *Pinus* species (Fig. 4 [↗](#), 50).

In addition, the root-zone pH gradient was often largely decoupled from root diameter, specific root length, root [N] and root tissue density (Table S10 [↗](#)). Although the quantity of root exudation may relate to root [N] and root tissue density (38 [↗](#), 51 [↗](#)), the quality (i.e., chemical components) of the exudation may not. Only some components of the total root exudates, such as organic acids, mediate the pH (52 [↗](#)). Thus, root-zone pH underpins a potential third gradient of the microbial-extended root economics space (Figs. 3 [↗](#), S3). This suggests that in addition to common root morphological and tissue chemical traits, the composition of the chemical compounds in the root exudates may also influence the rhizosphere processes through interactions with root-associated microbial communities (30 [↗](#)).

In summary, our study has revealed new and important relationships among common root economics traits, root-zone soil properties, and the functional composition of root-associated fungal and bacterial communities under the root economics framework. First, we identified a gradient of fungal guilds spanning from EcM to saprotrophic fungal dominance. This gradient was largely decoupled from the axis of root morphology, but was strongly coupled with root [N]. Second, our findings point to the importance of the root-zone pH gradient across the tree hosts. This pH gradient was largely decoupled from the common root traits, and closely correlated with the composition of fungal and bacterial communities. These novel gradients merge the functions of absorptive roots and their associated microbial communities, resulting in a microbial-extended root economics space that can improve our ability to predict rhizosphere and ecosystem functions (e.g., organic vs. mineral nutrient economics) using simple trait-based approaches.

Materials and Methods

Study site and species

We collected root branches of mature trees from a subtropical forest at Tianmu Mountain, Zhejiang Province (119.4394° E, 30.3255° N). The study site has an average annual temperature of 8.8–14.8 °C and annual precipitation of 1390–1870 mm. We established eleven 50 × 50 m² plots along an elevational gradient from 323 to 1268 m above sea level, with approximately 100-m of elevation between neighboring plots. In each plot, we sampled the roots of 6–8 of the most common tree species, with the number of tree individuals proportional to each species' relative abundance within the plot. This resulted in a total of 336 trees sampled for this study. We queried the tree species names against The Plant List (<http://www.theplantlist.org/>). We assigned the mycorrhizal type of each species based on the *FungalRoot* database (53), which assumes that mycorrhizal type is usually constant within a genus. **Table S1** provides the mycorrhizal type (arbuscular mycorrhizas vs. ectomycorrhizas), leaf habit (evergreen vs. deciduous), and phylogeny (gymnosperms vs. angiosperms) of each species.

Sampling and trait measurements

We collected root and rhizosphere soil samples in early October 2020. For each tree individual, we harvested distal root branches from four random locations within 2 meters of the stem at a soil depth of 0–20 cm. We followed the target root branches to the stem to confirm the identity of the species. Each root branch included at least the first five orders (31). We collected the 1st to 3rd-order roots from each root branch as absorptive roots and pooled all absorptive roots from the root branches of the same individual tree. We stored the absorptive roots and adhering soil in Ziploc bags on ice in a cooler and transported them to the laboratory within a few hours. We then froze the samples at -80 °C for later processing.

In the laboratory, we vigorously washed absorptive root samples in 50-mL Falcon tubes using a vortex mixer for 30 seconds. We then shook the roots vigorously with sterile forceps to remove all soil from the root surfaces, and collected all root segments. We used half of the root samples to study the microbial communities associated with root tissues, which may include the rhizoplane and intraradical communities. We used the remaining half of the root samples for root trait measurements. We centrifuged the muddy solution for 2 minutes and carefully removed the supernatants with pipettes. We collected the remaining soils to study the rhizosphere microbial communities.

To measure root traits, we first pooled and scanned root segments on a desktop scanner (Epson 12000XL, Epson America, Inc., San Jose, CA, USA). We then processed the scanned images using WinRHIZO (Regent Instruments, Inc., Ottawa, ON, Canada) to determine the average root diameter and total root length. We oven-dried the scanned roots at 65 °C for 72 hours and then weighed them. We calculated specific root length as the ratio of total root length to root dry weight of the scanned roots. We calculated root tissue density by dividing the root mass by the turgid tissue volume determined by WinRHIZO. We ground the oven-dried root samples and determined the root tissue nitrogen concentration using a FLASH 2000 CHNS/O Elemental Analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

For each selected tree, we also sampled the surrounding soil in the field to determine the soil properties. Because the root-adherent soil was not enough for soil assays, we collected soil within approximately 5 cm of the root surface to determine the root-zone soil properties. We measured the pH of air-dried soil using an FE28 pH meter (Mettler Toledo, Shanghai, China) with a soil solution ratio of 1:2.5. We determined gravimetric water content by oven-drying at 105 °C for 72 hours. We ground the oven-dried soil and determined the carbon and nitrogen concentrations using the same elemental analyzer as for roots.

Molecular methods

We extracted DNA from fresh root samples and root adherent soil samples using the PowerSoil DNA extraction kits (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. After DNA extraction, we amplified the *rbcL* and *matK* gene fragments in the root DNA to verify the plant species identity (54 [↗](#)). We amplified the ITS1 region of the fungal communities using the primer set ITS1F-ITS2 (CTTGGTCATTTAGAGGAAGTAA / GCTGCGTTCTTCATCGATGC). We amplified the V5-V7 regions of bacterial 16S rRNA using the primer set 799F-1193R (AACMGGATTAGATACCKG / ACGTCATCCCCACCTTCC) to study the bacterial communities. We included negative controls during DNA extraction and PCR amplification.

We sent successful PCR products for high-throughput sequencing on the Illumina Novaseq platform (2 x 250 bp), with approximately 50,000 reads per sample for the 16S region and approximately 100,000 reads for the ITS region. We processed the fungal amplicon sequences using QIIME2 (55 [↗](#)) and the bacterial amplicon sequences using USEARCH (56 [↗](#)). We merged and quality-filtered the paired reads, and then identified the amplicon sequence variants (ASVs). We assigned the taxonomy of each sequence using the RDP classifier (57 [↗](#)) with the UNITE databases for fungi (58 [↗](#)) and the RDP databases for bacteria (59 [↗](#)) at a 0.6 confidence threshold.

We used the *FungalTraits* database (34, version 1.2) to assign the functional guilds of fungal ASVs. If multiple lifestyles (i.e., guilds) were presented in the *FungalTraits* database, we used the primary lifestyle. We grouped the guilds into ectomycorrhizal (EcM) fungi, saprotrophic fungi, plant pathogenic fungi, fungi with other functions, and unspecified fungal ASVs (unknown taxonomy and unknown functions). We did not group fungal endophytes separately because their roles in plant nutrient acquisition are not fully understood. We calculated the relative abundance of different fungal guilds as the ratio of the corresponding read numbers to the total fungal read numbers.

In particular, we used two methods to estimate the relative abundance of AM fungi. First, we assigned Glomeromycotan ASVs from the ITS sequences as AM fungi and calculated the sequencing-based relative abundance across all samples (60 [↗](#)). Second, because the ITS region may not sufficiently amplify all AM fungal taxa (61 [↗](#)), we used qPCR methods to estimate the relative abundance of AM fungi, particularly for the rhizosphere soil samples. We estimated the qPCR-based relative abundance by quantifying the DNA copy number of AM fungi relative to the DNA copy numbers of all fungi, using primers AMG1F (ATAGGGATAGTTGGGGGCAT) and AM1 (GTTTCCCGTAAGGCGCCGAA) for AM fungi, and primers ITS5 (TCCTCCGCTTATTGATATGC) and ITS4 (GGAAGTAAAAGTCGTAACAAGG) for the whole fungal community (62 [↗](#)). Sequencing-based and qPCR-based relative abundance of AM fungi not greater than 100% were included in the subsequent statistical analyses.

We assigned the bacterial sequencing reads to bacterial guilds using the FAPROTAX database (63 [↗](#)). The guilds included bacterial taxa with the following functions: nitrification, nitrate reduction, nitrogen fixation, ureolysis, phototrophy, aromatic compound degradation, chitinolysis, fermentation, methylotrophy, other aerobic chemoheterotrophy (defined as aerobic chemoheterotrophy other than ligninolysis, chitinolysis, xylanolysis, cellulolysis, methanogenesis, methylotrophy, and aromatic compound degradation), bacteria with other functions (as one group), unspecified bacterial ASVs (unknown taxonomy and unknown functions). We calculated the relative abundance of different bacterial guilds as the ratio of the corresponding read numbers to the total bacterial read numbers.

Statistical analyses

We performed fungal and bacterial-related analyses separately because the relative abundance of a fungal guild is not directly comparable to the relative abundance of a bacterial guild. We first used marginal tests of distance-based redundancy analyses (dbRDA) to determine the

contributions of mycorrhizal type, plot elevation, absorptive root traits, and root-zone soil properties in shaping the composition of fungal or bacterial guilds across the rhizosphere and root tissue samples. We used the *dbrda* function in the R package *vegan*.

Next, we performed principal component analyses (PCAs) on the relative abundance of different fungal or bacterial guilds, as well as the corresponding root traits and root-zone soil properties across all tree individuals to construct the microbial-extended root economics space. We also performed PCAs at the species level by averaging each variable across the tree individuals of the same species. We used Horn's parallel analysis in the R package *paran* to determine the dimensionality of these PCAs (64 [↗](#)) and applied varimax rotation to the selected components with the principal function in the R package *psych* (65 [↗](#)). We used varimax rotation to simplify the interpretations of the most important axes in the root economics space (66 [↗](#)). We $\ln(x)$ transformed all data of root traits and soil properties to meet the normality requirement. To deal with zero values, we $\ln(x+0.001)$ transformed the relative abundance of fungal and bacterial guilds. We standardized the data used for principal component analyses by subtracting the mean and dividing by the standard deviation. We performed all statistics using R (version 4.1.1; R Foundation for Statistical Computing; www.r-project.org [↗](#)).

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Supervision: WC, SL

Writing—original draft: WC, RW

Writing—review & editing: MLM, CWF, TEJ, RTK

Competing interests

Authors declare that they have no competing interests.

Data and materials availability

All data included in this study will be available upon the acceptance of the manuscript.

Supplementary Materials for This PDF file includes:

Figs. S1 to S4

Tables S1 to S10

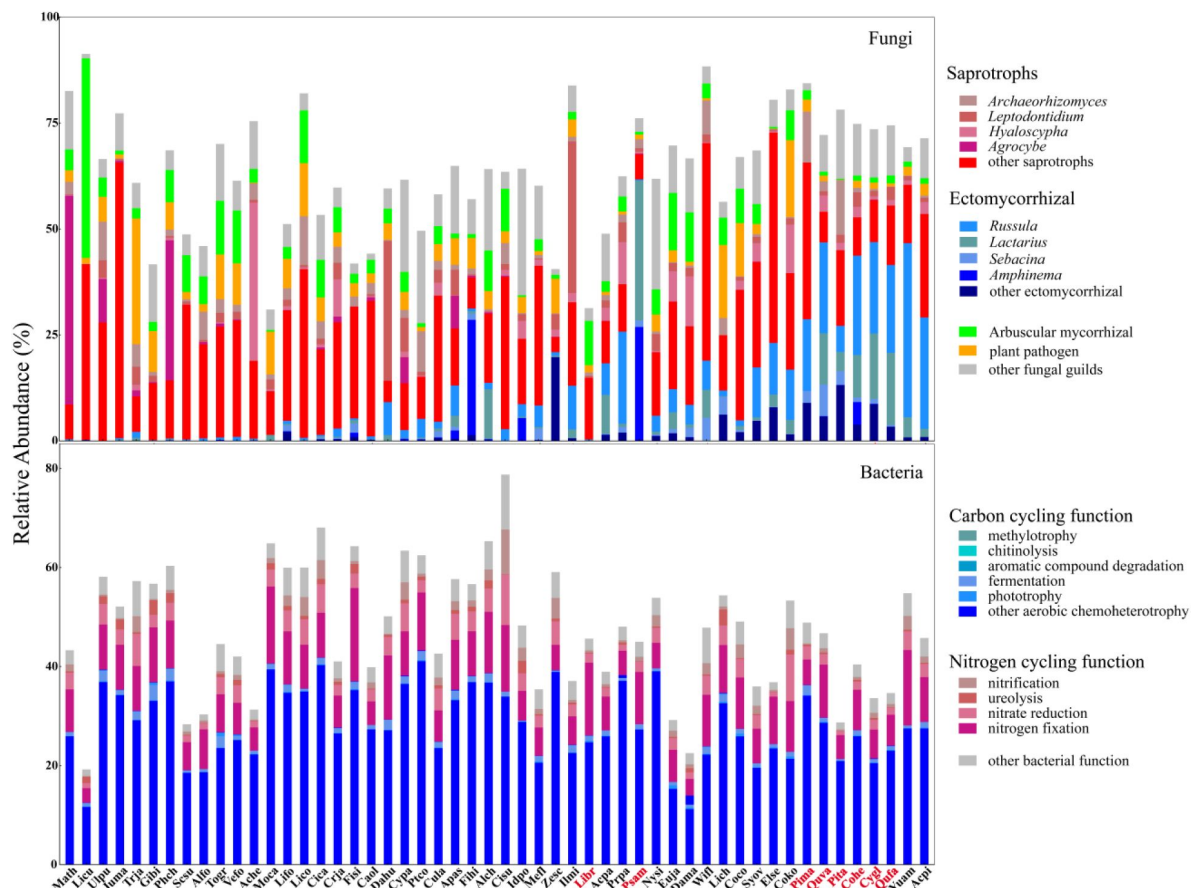


Fig. S1.

Compositions of fungal and bacterial guilds in the root tissue across 52 tree species.

The relative abundance of fungal (upper panel) and bacterial (lower panel) guilds is estimated based on sequencing reads. Compositions of the five most abundant ectomycorrhizal and saprotrophic genera are also shown. Abbreviations of ectomycorrhizal tree species are in red font while arbuscular mycorrhizal tree species are in black font. Complete scientific names of trees are provided in Table S1.

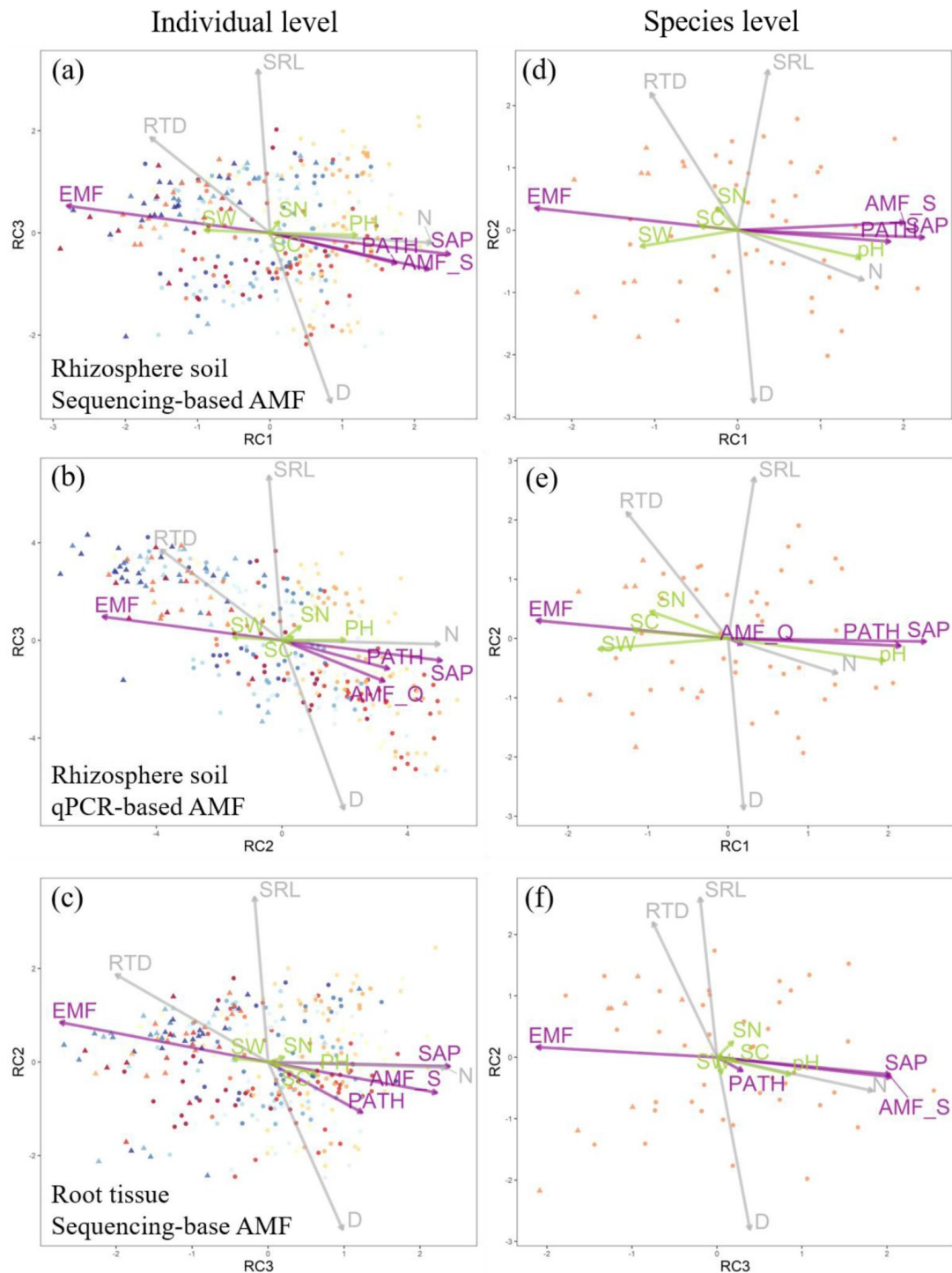


Fig. S2.

The gradient of fungal guilds (x-axis) in the root economics space.

Principal component analyses followed by varimax rotation were performed based on on root traits (grey), root-zone soil properties (green) and relative abundances of different fungal guilds (purple). The data are analyzed at either the individual (a-c) or the species level (d-f). The fungal communities are sampled from either rhizosphere soil (a-b, d-e) or root tissue (c, f). Within the rhizosphere fungal communities, the relative abundance of AM fungi is estimated using either the sequencing method (a, c, d, f, AMF_S) or the qPCR method (b, e, AMF_Q). Statistics of the PCAs are shown in Tables S4-S6. See [Fig. 1](#) for abbreviations of variables.

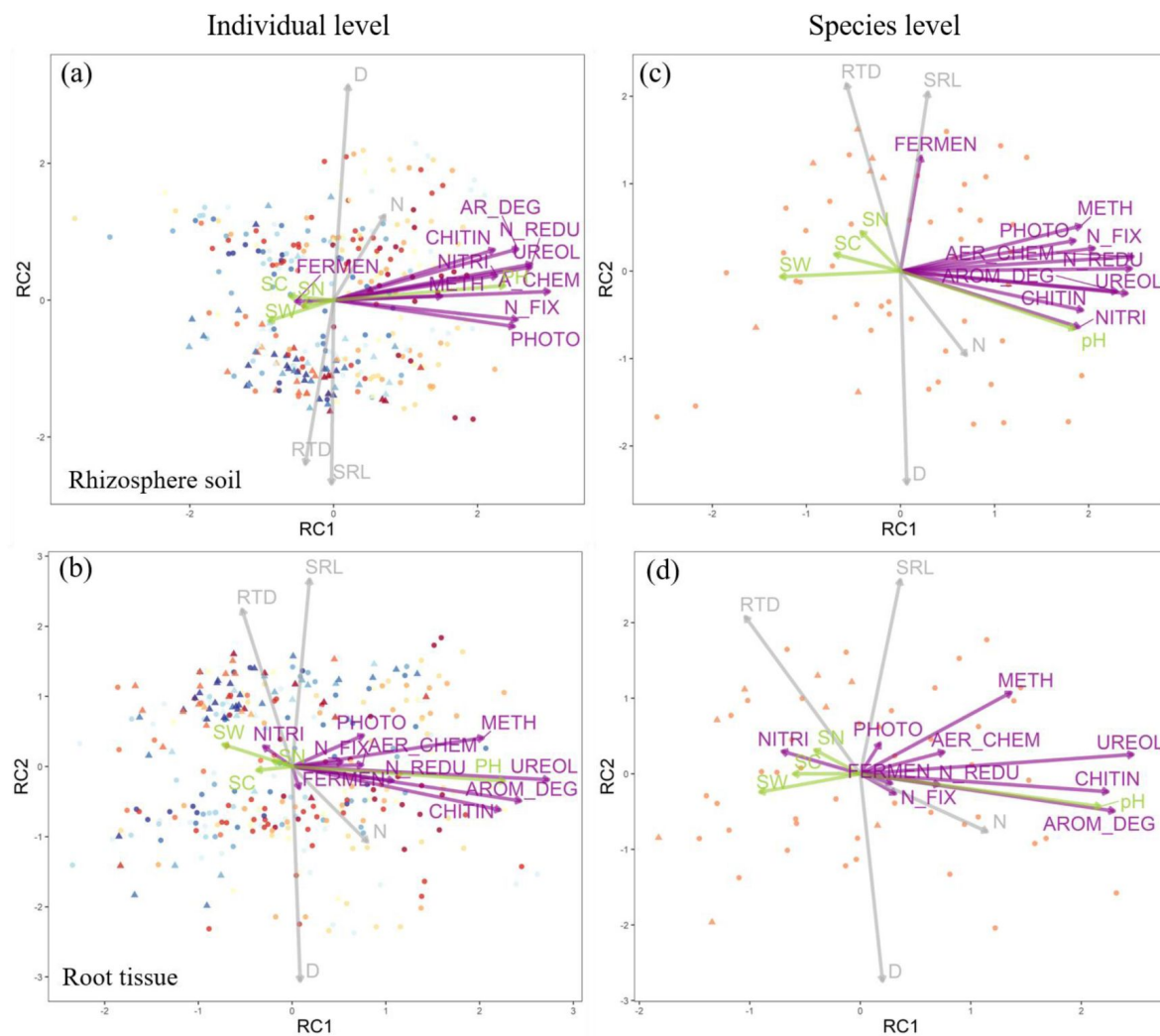


Fig. S3.

The gradient of bacterial guilds (x-axis) in the root economics space.

Principal component analyses followed by varimax rotation were performed based on root traits (grey), root-zone soil properties (green) and relative abundances of different bacterial guilds (purple). The data are analyzed at either the individual (a-b) or the species level (c-d). The bacterial communities are sampled from either rhizosphere soil (a, c) or root tissue (b, d). Statistics of the PCAs are shown in Tables S7-S8. See [Fig. 2](#) for abbreviations of variables.

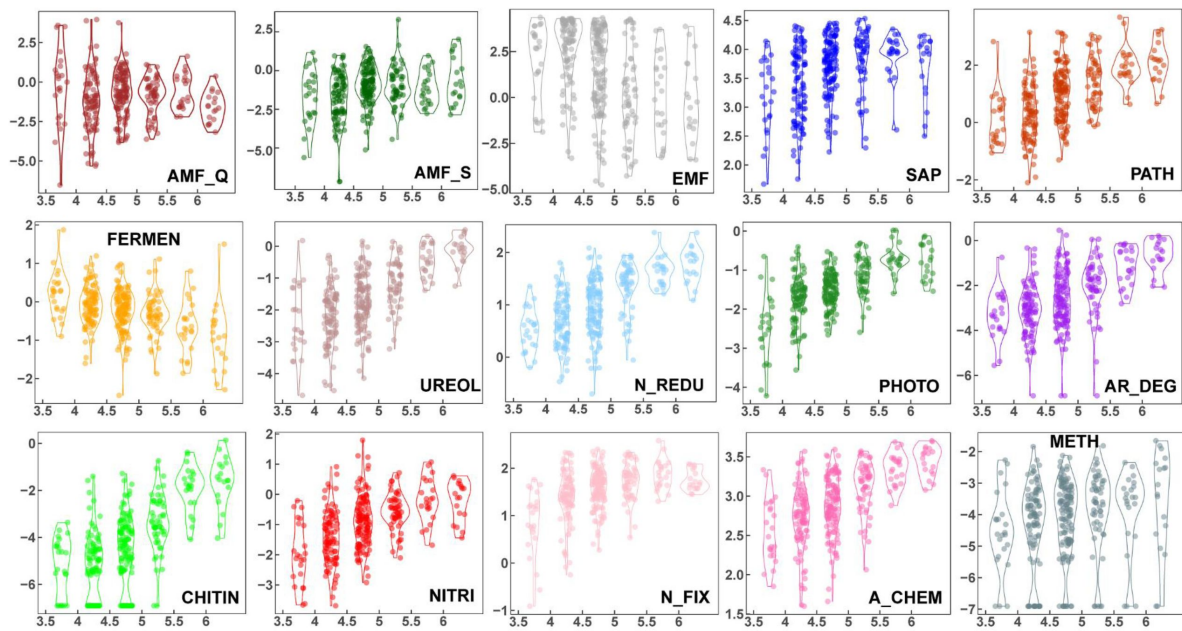


Fig. S4.

Relative abundance of different fungal and bacterial guilds along the rhizosphere pH intervals.

Relative abundance of AM fungi is estimated using either the qPCR method (AMF_Q) or the sequencing method (AMF_S). Data are $\ln(x+0.001)$ transformed. See [Figures 1](#) & [2](#) for abbreviations of fungal and bacterial guilds.

Species	Clade	Myc. type	Leaf habit	D	SRL	N	RTD	pH	SW	SC	SN
<i>Acer henryi</i>	angiosperms	AM	deciduous	0.37	19.7	23.5	0.48	4.69	116.9	135.8	6.4
<i>Acer palmatum</i>	angiosperms	AM	deciduous	0.16	59.0	14.7	0.90	4.38	76.3	100.4	7.5
<i>Acer pictum</i> subsp. <i>Mono</i>	angiosperms	AM	deciduous	0.25	28.3	NA	0.71	4.28	84.7	95.6	7.0
<i>Alangium chinense</i>	angiosperms	AM	deciduous	0.25	51.6	22.5	0.49	4.78	65.1	125.4	8.3
<i>Alniphyllum fortunei</i>	angiosperms	AM	deciduous	0.18	54.7	18.3	0.72	5.13	38.2	64.1	5.2
<i>Aphananthe aspera</i>	angiosperms	AM	deciduous	0.13	199.4	17.5	0.64	4.90	38.2	91.2	7.0
<i>Camellia oleifera</i>	angiosperms	AM	evergreen	0.30	24.5	16.6	0.81	4.44	63.5	99.8	6.8
<i>Cinnamomum camphora</i>	angiosperms	AM	evergreen	0.36	19.5	23.3	0.56	4.79	55.2	88.2	6.3
<i>Cinnamomum subavenium</i>	angiosperms	AM	evergreen	0.33	18.8	19.1	0.63	4.50	72.1	107.3	7.7
<i>Cornus controversa</i>	angiosperms	AM	deciduous	0.31	15.0	16.1	0.91	4.39	62.1	87.8	6.6
<i>Cornus kousa</i>	angiosperms	AM	deciduous	0.25	24.4	11.2	0.85	4.46	77.0	70.6	5.2
<i>Corylus heterophylla</i> var. <i>sutchuanensis</i>	angiosperms	EM	deciduous	0.14	67.3	16.8	1.10	4.38	81.6	103.0	6.7
<i>Cryptomeria japonica</i> var. <i>sinensis</i>	gymnosperms	AM	evergreen	0.38	16.7	16.1	0.56	4.58	60.1	115.1	7.4
<i>Cunninghamia lanceolata</i>	gymnosperms	AM	evergreen	0.46	12.1	15.2	0.54	4.41	60.9	118.2	7.7
<i>Cyclobalanopsis glauca</i>	angiosperms	EM	evergreen	0.18	45.3	14.0	1.03	4.56	82.1	115.8	8.0
<i>Cyclocarya paliurus</i>	angiosperms	AM	deciduous	0.17	53.4	23.1	0.96	4.85	54.4	79.6	5.8
<i>Dalbergia hupeana</i>	angiosperms	AM	deciduous	0.44	17.1	23.9	0.38	5.17	34.1	61.4	4.2
<i>Daphniphyllum macropodum</i>	angiosperms	AM	evergreen	0.32	24.5	19.3	0.54	4.35	105.9	136.4	8.8
<i>Eleutherococcus senticosus</i>	angiosperms	AM	deciduous	0.39	13.7	16.5	0.63	4.28	101.1	122.5	7.3
<i>Euscaphis japonica</i>	angiosperms	AM	deciduous	0.22	31.2	19.8	0.81	4.51	88.7	108.5	7.3
<i>Ficus hirta</i>	angiosperms	AM	deciduous	0.13	103.7	17.2	0.75	5.25	26.9	59.3	4.6
<i>Firmiana simplex</i>	angiosperms	AM	deciduous	0.38	15.1	10.0	0.59	6.01	46.7	61.8	4.4
<i>Ginkgo biloba</i>	gymnosperms	AM	deciduous	0.64	8.9	14.7	0.37	6.41	54.9	104.1	7.4
<i>Idesia polycarpa</i>	angiosperms	AM	deciduous	0.19	79.0	19.8	0.44	4.89	41.6	71.6	5.0
<i>Ilex micrococca</i>	angiosperms	AM	deciduous	0.14	9.8	17.4	0.64	4.28	73.1	93.1	6.2
<i>Juglans mandshurica</i>	angiosperms	AM	deciduous	0.16	52.8	19.2	0.93	5.99	52.0	74.5	5.4
<i>Liquidambar formosana</i>	angiosperms	AM	deciduous	0.25	25.5	13.5	0.92	5.36	61.4	78.1	5.6
<i>Liriodendron chinense</i>	angiosperms	AM	deciduous	0.49	19.8	15.3	0.34	5.67	38.1	33.9	2.4
<i>Lithocarpus brevicaudatus</i>	angiosperms	EM	evergreen	0.20	26.7	8.5	1.14	4.56	33.4	65.6	4.4
<i>Litsea coreana</i> var. <i>sinensis</i>	angiosperms	AM	evergreen	0.52	10.7	26.2	0.46	4.87	64.0	107.4	8.2
<i>Litsea cubeba</i>	angiosperms	AM	deciduous	0.40	19.4	34.3	0.41	5.68	58.7	90.7	5.9
<i>Machilus thunbergii</i>	angiosperms	AM	evergreen	0.42	18.3	21.5	0.39	5.14	40.3	63.7	4.3
<i>Meliosma flexuosa</i>	angiosperms	AM	deciduous	0.32	28.1	16.2	0.47	4.67	65.5	75.4	5.3
<i>Morus cathayana</i>	angiosperms	AM	deciduous	0.21	38.7	9.9	0.76	4.69	63.6	110.9	8.1
<i>Nyssa sinensis</i>	angiosperms	AM	deciduous	0.41	12.9	15.6	0.60	4.50	73.8	128.7	8.7
<i>Phoebe chekiangensis</i>	angiosperms	AM	evergreen	0.29	23.7	23.1	0.75	5.42	54.0	86.7	6.3
<i>Pinus massoniana</i>	gymnosperms	EM	evergreen	0.39	13.0	9.9	0.63	5.88	35.8	47.1	2.9
<i>Pinus taiwanensis</i>	gymnosperms	EM	evergreen	0.48	12.5	10.7	0.62	4.01	83.0	171.7	9.2
<i>Prunus padus</i>	angiosperms	AM	deciduous	0.17	49.0	16.5	0.91	4.46	62.4	121.0	9.3
<i>Pseudolarix amabilis</i>	gymnosperms	EM	deciduous	0.60	6.8	18.1	0.52	4.63	45.3	71.6	5.3
<i>Pterostyrax corymbosus</i>	angiosperms	AM	deciduous	0.19	57.4	27.6	0.61	4.61	99.9	124.7	11.5
<i>Quercus fabri</i>	angiosperms	EM	deciduous	0.17	40.8	12.4	1.07	4.41	80.8	104.0	7.2
<i>Quercus variabilis</i>	angiosperms	EM	deciduous	0.18	38.6	14.6	1.12	4.65	68.6	116.2	8.0
<i>Schima superba</i>	angiosperms	AM	evergreen	0.20	47.1	7.9	0.68	4.65	31.7	42.2	2.7
<i>Symplocos ovatilobata</i>	angiosperms	AM	evergreen	0.16	83.5	12.2	0.67	4.39	74.1	114.1	7.6
<i>Torreya grandis</i>	gymnosperms	AM	evergreen	0.74	6.4	23.6	0.38	4.99	60.2	104.6	7.4
<i>Trachelospermum jasminoides</i>	angiosperms	AM	evergreen	0.36	22.4	18.7	0.43	4.16	37.2	79.7	5.7
<i>Ulmus pumil</i>	angiosperms	AM	deciduous	0.16	65.8	13.8	0.77	5.21	35.2	93.7	6.1
<i>Vernicia fordii</i>	angiosperms	AM	deciduous	0.38	26.8	22.1	0.34	4.89	28.7	48.4	3.3
<i>Wisteria floribunda</i>	angiosperms	AM	deciduous	0.18	54.3	18.6	0.72	4.64	87.3	117.1	9.5
<i>Yulania amoena</i>	angiosperms	AM	deciduous	0.45	14.7	16.2	0.43	4.43	71.5	83.5	6.1
<i>Zelkova schneideriana</i>	angiosperms	AM	deciduous	0.15	61.2	11.6	0.88	4.57	37.4	68.0	5.9

Table S1.

Tree species in this study.

Both arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) tree hosts, either evergreen or deciduous, were selected. Only the first three order roots were used for morphology and tissue chemistry measurements. Values were averaged across individuals of the same species. Root-zone soil properties were measured within ca. 5 cm of root surface. D = root diameter (mm), SRL = specific root length (m g^{-1}), N = root nitrogen concentration (mg g^{-1}), RTD = root tissue density (g cm^{-3}), pH = root-zone soil pH, SW = gravimetric soil water content (%), SC = soil total carbon (mg g^{-1}), SN = soil total nitrogen (mg g^{-1}).

	Rhizosphere soil		Root tissue	
	Number of ASV	relative abundance	Number of ASV	relative abundance
Fungal guilds				
Total	2037	/	2019	/
SAP	594	42.87%	589	30.16%
EMF	257	18.97%	254	15.88%
PATH	111	4.37%	109	4.14%
AMF_S	166	0.66%	166	5.03%
Others	193	9.87%	192	10.00%
AMF_Q	/	1.58%	/	/
Bacterial guilds				
Total	2348	/	2352	/
AR_DEG	9	0.18%	9	0.35%
CHITIN	2	0.05%	2	0.08%
FERMEN	36	0.93%	36	0.80%
UREOL	17	0.30%	17	0.75%
N_FIX	24	5.13%	24	7.92%
NITRI	6	0.57%	6	1.29%
N_REDU	80	3.15%	80	3.05%
METH	5	0.03%	5	0.13%
PHOTO	11	0.27%	11	0.16%
A_CHEM	480	19.93%	480	27.54%
others	68	2.78%	72	3.85%

Table S2.

Summary of the root-associated fungal and bacterial communities.

Full names of fungal and bacterial guilds are provided in [Figs 1 & 2](#). The relative abundance of AM fungi is estimated using either the qPCR method (AMF_Q) or the sequencing method (AMF_S).

	Fungi			Bacteria		
	var%	F	P	var%	F	P
Elevation	0.9%	3.5	0.033	2.0%	7.3	0.001
Mycorrhizal type	13.8%	53.2	0.001	0.5%	1.7	0.13
Root diameter	0.2%	0.6	0.58	0.2%	0.7	0.52
Specific root length	0.2%	0.7	0.56	0.2%	0.6	0.63
Root [N]	0.8%	3.0	0.05	0.6%	2.4	0.067
Root tissue density	0.2%	0.7	0.52	0.2%	0.7	0.54
Soil pH	2.6%	9.9	0.001	13.4%	50.0	0.001
Soil water content	0.8%	3.0	0.05	0.2%	0.7	0.56
Soil total carbon	0.7%	2.6	0.07	0.4%	1.4	0.22
Soil total nitrogen	0.2%	0.8	0.47	0.4%	1.4	0.20
Full model	40.7%	21.0	0.001	29.1%	12.5	0.001

Table S3.

Distance-based redundancy analysis (dbRDA) to determine the predictor variables that significantly influenced the functional compositions of fungal and bacterial communities within the roots.

Predictor variables include elevation, host mycorrhizal type, absorptive root traits, and root-zone soil properties. Results show marginal tests based on the Manhattan distance matrix, where var% indicates the relative contributions of predictor variable to fungal and bacterial guild dissimilarity. The relative abundance of AM fungi is estimated by ITS sequencing in this table. Significant statistics ($P < 0.05$) are indicated in bold.

Component	Rhizosphere soil			Root tissue		
	RC2	RC1	RC3	C1	C2	C3
% variance	27%	23%	18%	26%	19%	18%
EMF	0.22	-0.82	0.16	0.23	0.23	-0.73
SAP	-0.31	0.72	-0.12			0.64
PATH	-0.44	0.51	-0.17	-0.48	-0.29	0.33
AMF	-0.21	0.64	-0.21	-0.19	-0.18	0.59
D		0.25	-0.96		-0.95	0.26
SRL			0.93		0.94	
N	0.21	0.65		0.12		0.62
RTD		-0.48	0.54		0.50	-0.53
PH	-0.56	0.35		-0.60		0.17
SW	0.82	-0.26		0.85		-0.12
SC	0.94			0.94		
SN	0.94			0.93		

Table S4.

Statistics of the root economics space incorporating fungal guilds across individual trees.

Shown are the proportion of variance and loadings of the relative abundance of fungal guilds, root traits, and root-zone soil properties in the most significant components of the principal component analyses followed by varimax rotation. The relative abundance of AMF is estimated by ITS sequencing. Loadings between -0.1 and 0.1 are not shown. Full names of fungal guilds, root traits, and soil properties are provided in [Figure 1](#).

	Rhizosphere soil			Root tissue		
	RC1	RC3	RC2	RC1	RC2	RC3
% variance	26%	25%	21%	29%	20%	18%
EMF	-0.85	0.11	0.12	0.34		-0.73
SAP	0.78	-0.24				0.70
PATH	0.64	-0.32		-0.61		0.10
AMF	0.70			-0.22	-0.11	0.70
D			-0.96		-0.96	0.13
SRL	0.13		0.89		0.89	
N	0.53	0.46	-0.28	0.22	-0.19	0.63
RTD	-0.37	0.10	0.77	0.22	0.76	-0.26
PH	0.52	-0.49	-0.15	-0.62		0.30
SW	-0.41	0.80		0.90		
SC	-0.16	0.94		0.94		
SN		0.94	0.13	0.89		

Table S5.

Statistics of the fungal-extended root economics space at the species level.

Shown are the proportion of variance and loadings in the most significant components of the principal component analyses followed by varimax rotation. The relative abundance of fungal guilds, root traits and soil prosperities are averaged across individuals of the same species. The relative abundance of AM fungi is estimated by ITS sequencing. Loadings between -0.1 and 0.1 are not shown. Full names of fungal guilds, root traits, and soil properties are provided in [Figure 1](#).

Fungi	Individual level			Species level		
	RC1	RC2	RC3	RC1	RC2	RC3
% variance	27%	21%	18%	29%	21%	21%
EMF	0.28	-0.79	0.14	-0.82		0.11
SAP	-0.37	0.70	-0.11	0.85		
PATH	-0.49	0.47	-0.16	0.74	-0.12	
AMF		0.45	-0.23		0.13	
D		0.27	-0.96			-0.99
SRL			0.93	0.11		0.93
N	0.16	0.69		0.47	0.63	-0.20
RTD		0.53	0.51	-0.44		0.73
PH	-0.60	0.28		0.67	-0.31	-0.13
SW	0.83	-0.22		-0.56	0.70	
SC	0.94			-0.41	0.86	
SN	0.93			-0.33	0.87	0.16

Table S6.

Statistics of the fungal-extended root economics space with qPCR-based relative abundance of AM fungi.

Only the relative abundance of fungal guilds in the rhizosphere were included in the analyses. Shown are the proportion of variance and loadings in the most significant components of the principal component analyses followed by varimax rotation at both individual and species levels. Loadings between -0.1 and 0.1 are not shown. Full names of fungal guilds, root traits, and soil properties are provided in [Figure 1](#).

% variance	Rhizosphere soil				Root tissue				
	RC1	RC3	RC2	RC4	RC1	RC3	RC4	RC2	RC5
	33%	16%	14%	8%	18%	16%	15%	14%	11%
AR_DEG	0.79		0.23		0.78	-0.14	0.19	-0.16	0.25
CHITIN	0.69	-0.13	0.23	-0.15	0.71	-0.22	0.22	-0.20	
FERMEN	-0.16			0.86			0.81		
UREOL	0.84	-0.16	0.15		0.87				0.19
N_FIX	0.78			0.17	0.17	-0.15	0.79		0.34
NITRI	0.70	-0.23	0.11						0.87
N_REDU	0.84	-0.20	0.16	0.20	0.34	-0.13	0.42		0.74
METH	0.46			0.57	0.65			0.13	
PHOTO	0.77	-0.21	-0.12	-0.21	0.24		0.75	0.14	-0.19
A_CHEM	0.92			0.16	0.24		0.69		0.41
D			0.96					-0.98	
SRL			-0.83				-0.12	0.85	0.14
N	0.22	0.13	0.38	0.27	0.26	0.15	-0.27	-0.34	0.39
RTD	-0.12		-0.74		-0.17		0.15	0.72	-0.16
PH	0.73	-0.28		-0.41	0.72	-0.37	0.17		-0.15
SW	-0.28	0.83			-0.23	0.83		0.10	-0.11
SC	-0.19	0.94		0.12	-0.12	0.95			
SN	-0.13	0.96				0.96			

Table S7.

Statistics of the root economics space incorporating bacterial communities across individual tree hosts.

Shown are the proportion of variance and loadings of the relative abundance of bacterial guilds, root traits, and root-zone soil properties in the most significant four or five components of the principal component analyses followed by varimax rotation. Loadings between -0.1 and 0.1 are not shown. Full names of bacterial guilds, root traits, and soil properties are provided in **Figure 2**.

	Rhizosphere soil			Root tissue				
	RC1	RC3	RC2	RC1	RC3	RC4	RC2	RC5
% variance	39%	16%	16%	19%	16%	14%	14%	9%
AR_DEG	0.88	0.12		0.80	-0.21	0.38	-0.17	
CHITIN	0.74	-0.17	-0.17	0.78	-0.20			0.15
FERMEN		0.21	0.50		0.16			0.72
UREOL	0.92			0.86		0.22		
N_FIX	0.78			0.11	-0.18	0.63		0.50
NITRI	0.72	-0.25	-0.24	-0.25	0.17	0.80	0.10	-0.20
N_REDU	0.93	-0.18		0.25		0.89		
METH	0.73		0.20	0.47	0.12	-0.13	0.37	
PHOTO	0.71	-0.37	0.13		0.12		0.14	0.68
A_CHEM	0.94			0.26	-0.10	0.68	0.10	
D			-0.93				-0.95	
SRL	0.11		0.78	0.13			0.89	-0.12
N	0.27	0.47	-0.37	0.40	0.51		-0.27	-0.35
RTD	-0.22		0.82	-0.36			0.72	0.37
PH	0.70	-0.36	-0.25	0.75	-0.43		-0.15	
SW	-0.49	0.73		-0.32	0.79	-0.19		0.25
SC	-0.27	0.92		-0.21	0.91	-0.10		0.11
SN	-0.16	0.93	0.17	-0.14	0.92		0.11	0.10

Table S8.

Statistics of the bacterial-extended root economics space at the species level.

Shown are the proportion of variance and loadings in the most significant components of the principal component analyses followed by varimax rotation. The relative abundance of bacterial guilds, root traits and root-zone soil prosperities are averaged across individuals of the same species. Loadings between -0.1 and 0.1 are not shown. Full names of fungal guilds, root traits, and soil properties are provided in **Figure 2**.

Variables	F	P		
Elevation	3.567	<0.001		
Species	2.750	<0.001		
Elevation * Species	1.224	0.15		
Species effect in each plot	F	P	pH _{Min}	pH _{Max}
S1 (323m)	5.788	0.008	4.45	6.25
S2 (466m)	15.724	<0.001	3.89	7.26
S3 (538m)	3.284	0.018	4.16	7.54
S4 (623m)	13.114	<0.001	3.58	6.37
S5 (714m)	4.338	0.02	3.96	6.29
S6 (809m)	23.233	<0.001	3.79	7.20
S7 (935m)	1.524	0.25	3.49	4.89
S8 (1060m)	2.638	0.054	3.68	5.59
S9 (1117m)	1.199	0.35	3.83	5.25
S10 (1190m)	1.797	0.19	3.55	4.68
S11 (1268m)	0.940	0.45	3.98	4.64

Table S9.

Influence of elevation and tree species on rhizosphere pH.

Bold values indicate significant effects at $P < 0.05$.

	PC1	PC2	PC3
Eigenvalue	2.39	1.03	0.98
% Variance	47.7	20.6	19.5
Diameter	0.63	-0.21	
Specific root length	-0.54	0.44	0.10
Nitrogen concentration	0.26	0.81	
Root tissue density	-0.48	-0.33	0.16
Root-zone soil pH	0.13		0.99

Table S10.

Principal component analysis on common root traits and root-zone soil pH.

Shown are the eigenvalues, proportion of variance and loadings of the first three principal components, without loadings between -0.1 and 0.1.

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Reviewer #1 (Public Review):**Summary:**

In this study, Wu et al. investigated the microbiome in the rhizosphere and roots of plant species along an elevational gradient. They found that: (i) plants with higher root nitrogen ("fast" strategy) were more likely to be associated with saprotrophic fungi, plant pathogenic fungi, and AM fungi, but plants with lower root nitrogen ("slow" strategy) were more likely to be associated with ectomycorrhizal fungi; (ii) bacterial functional guilds were associated with root-zone pH but not root traits.

Strengths:

This study is novel in the sense that it revealed the associations between microbiome and trait dimensions of plants. This has been rarely explored even though we acknowledge the importance of plant-microbe interactions.

Weaknesses:

The authors tried to include the relative abundances of bacterial and fungal guilds into the root economics framework, which I disagree with because they are just associated with the root economics framework. The title also states that the authors' aim is to link microbial functional guilds to root economics. Therefore, I would suggest that the analyses should be redone to elaborate on the relationships between microbiome and root functional traits.

Below I provide some critiques and comments that outline my concerns and provide recommendations to hopefully improve the current manuscript.

-Figures 2 and 3: The authors included soil properties, relative abundances of bacterial or fungal guilds, and root traits in the root economics spectrum. However, soil properties and relative abundances of bacterial or fungal guilds are not root traits, they are just associated with root traits. These bacterial or fungal guilds are the consequence of root traits. Also, the authors did not elaborate on the root trait dimensions of the plants. The only trait dimension they discussed is the "fast-slow" axis. Therefore, I would suggest the authors first analyze the trait dimensions of plants by only using the root traits (PCA), and then explore how the soil properties and relative abundances of bacterial or fungal guilds are associated with the trait dimensions (e.g., envfit in the vegan package).

-When exploring the associations between microbial functional guilds and root traits, it is unnecessary to analyze the bacterial and fungal functional guilds separately. The bacterial and fungal functional guilds can be included in the same models, and their relative importance and patterns can be compared.

-For fungi, the authors used FUNGuild to infer functional guilds from taxonomy. qPCR was also performed to validate the results of AMF. This is fantastic. For bacteria, the authors used FAPROTAX to infer functional guilds from taxonomy. However, archaea are also considered in some functions in FAPROTAX. For example, both bacteria (ammonia-oxidizing bacteria) and archaea (ammonia-oxidizing archaea) play critical roles in nitrification. I would assume the authors have removed archaea from the dataset because they stated that the functions of bacteria are inferred from FAPROTAX. Therefore, the importance of nitrification might be underestimated.

-Key methodological details are missing. First, maps of the sampling site and plots are missing. It would be great if the authors provided maps showing the location of the sampling site and the spatial distribution of the 11 plots. Second, in lines 304-306 the authors claimed that they sampled the most common species in the plots, but they did not provide the coverage or relative abundances of plant species in the plots.

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Reviewer #2 (Public Review):

Summary:

The authors aimed to determine to what extent root morphology, chemistry, and soil characteristics explained the relative abundance of functional groups of bacteria and fungi associated with roots. To do so, they sample roots and rhizospheric soil of trees along an elevation gradient. This type of work is common in the field of microbial ecology. The main novelties I see are two: a) a focus on the functional groups of bacteria and fungi rather than just taxonomic abundance. I think this approach is valuable because it provides information about the potential functions of these microorganisms; b) using the root economic spectrum to frame the findings. The root economic spectrum reflects a gradient along which plant roots can be allocated from 'short-lived that provide fast investment return' to 'long-lived that provide a slow investment return'. It is logical to expect (as the authors did) that variation along this gradient will be an important factor in explaining the variation in functional groups.

Strengths:

The main strength is using the root economic spectrum as a framework to interpret the data. There are countless studies addressing variation in the relative abundance of microbial communities along environmental gradients which tend to be more descriptive. I think using this framework advances the field by suggesting that while the root economic spectrum exists it is not a very important explanatory variable to predict changes in functional diversity. I

also think the authors use state-of-the art methods to collect and process the sample (i.e. to obtain the data).

Weaknesses:

The main weakness is with the presentation of statistical methods as it currently stands. The authors use distance-based redundancy analysis as the main statistical method. However, my understanding is that this method is not advised for a relative abundance of communities. At least not with Euclidean distances which is the default option of the functions `dbrda` in `vegan`. The use of this distance would group together communities with no species in common as close to each other (which is an incorrect interpretation). I think the authors should specify what distance they use. My guess is that they actually used bray-curtis in which case this weakness does not apply. However, as it stands it is not specified what metric they use and if they indeed use Euclidean distances it may lead to inaccurate conclusions. In addition, they also mention they use PCA on the relative abundance of functional groups. By definition, PCA is also based on Euclidean distances, which gives a similar problem as `dbrda`. Thus, I encourage the authors to use bray-curtis distance and specify it in the text.

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Reviewer #3 (Public Review):

Summary:

In this study, the authors collected a large set of data on root traits and root-associated microbes in the root endosphere and rhizosphere in order to integrate these important organisms in the root economics spectrum. By sampling a relatively large set of species from the subtropics along an elevation gradient, they tested whether microbial functions covary with root traits and root trait axes and if so, aimed to discuss what this could tell us about the (belowground) functioning of trees and forests.

Strengths:

The strengths of this study lie mostly in the impressive dataset set the authors compiled: they sampled belowground properties of a relatively large number of tree species from an understudied region: i.e., the subtropics, where species-level root data are notoriously scarce. Secondly, their extensive sampling of associated microbes to integrate them in the root economics space is an important quality, because of the strong associations between roots and fungi and bacteria: soil microbes are directly related to root form (e.g., mycorrhizal fungi and root diameter and SRL), and function (e.g., taking up soil nutrients from various sources). Thirdly, the PCA figures (Figures 2 and 3) look very nice and intuitive and the paper is very well written.

Weaknesses:

That said, this study also has several methodological weaknesses that make the results, and therefore the impact of this study difficult to evaluate and interpret.

(1) Design: The design of this study needs further explanation and justification in the Introduction and Methods sections in order to understand the ecological meaning of the results. Root traits and microbial community composition differ with their environment, and therefore (likely) also with elevation. Elevation is included in the redundancy analysis as a main effect, but without further environmental information, its impact is not ecologically meaningful. What is the rationale for including an elevation gradient in the design and as a main effect in the analyses? Do environmental conditions vary across altitudes and how, and if so, how would this impact the data?

What is the rationale behind sampling endosphere and rhizosphere microbial communities - why do both? And why also include pathogens - what are their expected roles in the RES?

What do we know about this already? The introduction needs a more extensive literature review of these additional variables that are included in the analyses.

(2) Units of replication and analysis in the model: What are the units of replication and analyses, e.g., how many trees were sampled per species, how many species or trees per elevation, and how many plots per elevation? Were all 11 plots at different elevations and if so, which ones? The level of analysis for the redundancy analyses is not entirely clear: L. 404 mentions that the analyses were done 'across the rhizosphere and root tissue samples', but is that then at the individual-tree level? If so, it seems that these analyses should then also account for dependencies between trees from the same species and phylogeny (as (nested) covariates or random factors). With the information provided, I cannot tell whether there was sufficient replication for statistical interpretations.

(3) PCA: The results of the parallel analyses are not described: which components were retained? Because the authors aim to integrate microbial functions in a root economics space, I recommend first demonstrating the existence of a root economics space across the 52 subtropical species before running a PCA that includes the microbial traits. The PCA shown in this study does not exactly match the RES and this could be because traits of these species covary differently, but may also simply result from including additional traits to the PCA.

Also, the PCA's shown are carried out at the individual-tree level. I would recommend, however, including the species-level PCA's in the main text, because the individual-level PCA may not only reflect species-inherent ecological strategies (that e.g., the RES by Bergmann et al. 2020 describe) but also plasticity (Figures 2 and 3 both show an elevation effect that may be partly due to plasticity). While the results here are rather similar, intraspecific differences in root traits may follow different ecological principles and therefore not always be appropriate to compare with an interspecific RES (see for example Weemstra & Valverde-Barrantes, 2022, *Annals of Botany*).

I could not deduce whether tree species in the "fungal PCA" (Figure 2) were assigned as AM or EcM based on Table 1, or based on their observed fungal community composition. In the former case, the fungal functional guild gradient (from EcM to saprotrophs and AM) is partially an artificial one, because EcM tree species are not AM species (according to Table 1) and therefore, by definition, constitute a tradeoff or autocorrelation. And, as the authors also discuss, AM tree species may host EcM fungal species. Before I can evaluate the ecological meaning of PC1, and whether or not it really represents a mineral/organic nutrient gradient, information is needed on which data are used here.

I do not agree with the term 'gradient of bacterial guilds' (i.e., PC1 in Figure 3). All but 1 bacterial 'function' positively loaded on PC1 and 'fermentation' was only weakly negatively correlated with PC1. I do not think this constitutes a 'bacterial gradient'.

(4) Soil samples: Were they collected from the surrounding soil of each tree (L. 341), or from the root zone (L. 110). The former seems to refer to bulk soil samples, but the latter could be interpreted as rhizosphere soils. It is therefore not entirely clear whether these are the same soil samples, and if so, where they were sampled exactly.

Aims:

The authors aimed to integrate endospheric and rhizospheric microbial and fungal community composition in the root economics space. Owing to statistical concerns (i.e., lacking parallel analysis results and the makeup of the PCs (AM versus EcM classification), I am not sure the authors succeeded in this. Besides that, the interpretation of the axes seems rather oversimplified and needs some consideration.

Root N is discussed as an important driver of fungal functional composition. Indeed, it was one of the significant variables in the redundancy models predicting microbial community composition, but its contribution to community composition was small (2 - 3 %), and the

mechanistic interpretation was rather speculative. Specifically, the role of root N in root (and tree) functioning remains highly uncertain: the link with respiration and exudation is increasingly demonstrated but its actual meaning for nutrient uptake is not well understood (Freschet et al. 2021. *New Phytologist*). If and how root economics (represented by root N) and the fungal-driven nutrient economy (EcM versus AM, saprotrophs) can indeed be integrated into a unified framework (L. 223 - 224) seems a relevant question that is worth pursuing based on this paper, but in my opinion, this study does not clearly answer it, because the statistical analyses might need further work (or explanation) and underlying mechanisms are not well explained and supported by evidence.

In addition, the root morphology axis was indeed independent of the "fungal gradient", but this is in itself not an interesting finding. What is interesting, but not discussed is that, generally, AM species are expected to have thicker roots than EcM tree species (Gu et al. 2014 *Tree Physiology*; Kong et al. 2014 *New Phytologist*). I am therefore curious to see why this is not the case here? Did the few EcM species sampled just happen to have very thick roots? Or is there a phylogenetic effect that influences both mycorrhizal type and root thickness that is not accounted for here (Baylis, 1975; Guo et al., 2008 *New Phytologist*; Kubisch et al., 2015 *Frontiers in Plant Science*; Valverde-Barrantes et al., 2015 *Functional Ecology*; 2016 *Plant and Soil*)?

I also do not agree with the conclusion that this integrated framework 'explained' tree distributions along the elevation gradient. First of all, it is difficult to interpret because the elevation gradient is not well explained (e.g., in terms of environmental variation). Secondly, the framework might coincide with the framework, but the framework does not explain it: an environmental gradient probably underlies the elevation gradient that may be selected for species with certain root traits or mycorrhizal types, but this is not tested nor clearly demonstrated by the data. It thus remains rather speculative, and it should be more thoroughly explained based on the data observed. Similarly, I do not understand from this study how root traits like root N can influence the abundance of EcM and pathogenic fungi (L. 242 - 243). Which data show this causality? It seems a strong statement, but not well supported (or explained).

Impact:

The data collected for this study are timely, valuable, and relevant. Soilborne microbes (fungi and bacteria; symbionts and pathogens) play important roles in root trait expressions (e.g., root diameter) and below-ground functioning (e.g., resource acquisition). They should therefore not be excluded from studies into the belowground functioning of forests, but they mostly are. This dataset therefore has the potential to improve our understanding of this subject. Making these data publicly available in large-scale datasets that have recently been initiated (e.g., FRED) will also allow further study in comparative (with other biomes) or global (across biomes) studies.

Technically, the methodology seems sound, although I lack the expertise to judge the Molecular Methods (L. 349 - 397). However, owing to some statistical uncertainties mentioned above (that the authors might well clarify or improve) and the oversimplified discussion, I am hesitant to determine the impact of the contents of this work. Statistical improvements and/or clearer explanation/justification of statistical choices made can make this manuscript highly interesting and impact, however.

Context:

As motivated above, I am not sure to what extent the EcM - AM/saprotroph presents a true ecological tradeoff. However, if it does, this work would fit very well in the context of the mycorrhizal-associated nutrient economy (Phillips et al. 2013 *New Phytology*). This theory postulates that EcM trees generally produce low-quality litter (associated with 'slow traits') that can be more readily accessed by EcM but not AM fungi, thereby slowing down nutrient cycling rates at their competitive advantage, and vice versa for AM tree species. This study

did not aim to test the MANE, so it was beyond its scope to study litter quality, and the number of EcM and AM species was unbalanced (8 EcM versus 44 AM species): nonetheless, the denser roots of EcM species and higher root N of AM species indicates that the MANE may also apply to this subtropical forest and may be an interesting impetus for future work on this topic. It might also offer one way to bridge the root economics space and the MANE.

What I also found interesting is the sparse observations of EcM fungal taxa in the root endosphere of species typically identified as AM hosts (L. 212 - 214). While their functionality remains to be tested (fungal structures in the endosphere were not studied here), this observation might call for renewed attention to classifying species as AM, EcM, or both.

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Reviewer #4 (Public Review):

Summary:

Recent progress in root economics has revealed global-scale axes of covaried root traits that reflect various root resource acquisition strategies. These covariance patterns are powerful tools for understanding root functional diversity. However, roots do not function in isolation for below-ground resource acquisition. Rather, symbiotic fungi and rhizosphere microorganisms often collaborate with plant roots, forming a root-microbial-soil continuum. This study seeks to provide novel insights into this continuum by extending the existing framework of root economics to include the structures of root-associated microorganisms. I find this topic highly relevant. Considering the role of soil microorganisms is undoubtedly crucial for a more comprehensive understanding of below-ground resource strategies.

Major comments:

A key finding of this study is a relationship between root N and the tendency for roots to associate with particular types of mycorrhizal associations (Line 27, Fig. 2). The authors concluded that this indicates "a linkage from simple root traits to fungal-mediated carbon nutrient cycling" (line 27) and integrates "microbial functions into the root economics framework," (line 32). If substantiated, this correlation could represent a significant discovery about the connection between root functional traits and root-associated fungi. It suggests that low root N, indicative of low metabolic activity within the root economics framework, is linked with forming EcM associations. However, I am not fully convinced this is the case based on the current data presentation and interpretation.

First, there is no biological interpretation of this relationship between root N and mycorrhizal type. It merely noted that root N is indicative of root metabolic activity, and thus by relating root N to fungal composition, "the trait-related root economics and fungal-driven nutrient economics may be integrated into a unified framework" (lines 221-224). Why would roots with low N and low metabolic activity tend to favor EcM associations? What are the potential mechanisms? Biological interpretation is essential for understanding whether a statistical correlation reflects a causal and meaningful relationship or is coincidental.

I am also concerned that this relationship may be spurious, especially when it lacks biological interpretation. EcM is underrepresented in this study (8 EcM species, of which more than half are conifers and oaks vs. 44 AM) and seems to cluster at higher elevations (line 231). Thus, the tree species/individual data points are not independent, but phylogenetically and geographically clustered. The unique properties at higher elevations (e.g., distinct plant community structures, low levels of mineral N) may drive both the lower root N and the prevalence of EcM associations. This scenario aligns with the observation that at higher elevations, AM roots also exhibited low root N (Line 231). In this case, root N may not directly relate to mycorrhizal type but is characteristic of certain locations (or closely related species), and it would be misleading to suggest that low root N/metabolic activity, a proxy in fast-slow

root economics, is directly linked to the preference for a particular mycorrhizal type (lines 27-28, 220 - 224). In summary, because the studied tree species appear to be clustered both phylogenetically and geographically, these factors need to be carefully taken into account in the statistical analysis and data interpretation to understand the underlying causes of the apparent relationship and prevent overinterpretation. I also recommend, if possible, providing a visual presentation of the geographical and phylogenetic distribution of the studied tree species.

That being said, this dataset is undoubtedly valuable in revealing the shifts in the compositional structures of root-associated soil microorganisms. However, integrating the traits of microbial composition to root trait economics would require more caution and careful examination of the potential driving causes.

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