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Research Article

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Posted Date: August 6th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4699468/v1>

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Rare taxa in the rhizosphere primarily influence nitrogen dynamics in plantations in northeastern China, with minimal impact on carbon and phosphorus

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Abstract

Aims: Rhizosphere effects refer to the alterations in soil properties resulting from the presence of plant roots. This study assessed the influence of rhizosphere microbial communities on the rhizosphere effects of carbon, nitrogen and phosphorus content.

Methods: we examined the rhizosphere effects of carbon, nitrogen and phosphorus in plantation forests of *Acer saccharum* (Ace), *Larix gmelinii* (Lar), *Pinus sylvestris* var. *mongolica* (Pin) and *Populus* sp. (Pin). We studied root functional traits and rhizosphere microbial communities and categorized rhizosphere microbes into two sub-communities, rare and abundant taxa, based on their relative abundance. Furthermore, we analyzed the chemical properties of rhizosphere and bulk soils and quantified the rhizosphere effects in the study areas.

Results: The results revealed that in Pop trees, the rhizosphere effects of total nitrogen and alkali-hydrolyzable nitrogen were significantly higher compared to other tree species. Abundant taxa assembly was dominated by deterministic processes, whereas rare taxa were more influenced by stochastic processes. The rhizosphere rare taxa exhibited a stronger correlation with root functional traits in comparison to the abundant taxa. Both the Random Forest Model and Partial Least Squares Path Model suggested that rhizosphere rare taxa played a crucial role in the rhizosphere effect of nitrogen. The rhizosphere effects of carbon and phosphorus could not be accounted for by the rhizosphere microbial community properties.

Conclusion: These results collectively suggest that plant roots may regulate the composition of the rhizosphere microbial sub-community to meet their nitrogen requirements, underscoring the

importance of rare rhizosphere taxa in plant nutrient uptake.

Keywords: Root functional traits; Rhizosphere effects; Rare microbes; Microbial community; Plant-soil interactions.

Introduction

The rhizosphere effects, commonly referred to as alterations in microbial community structure and soil properties brought about by plant roots, are primarily governed by the functional traits of plant roots (Chao et al. 2023; Han et al. 2020; Phillips and Fahey 2006). Recent studies have introduced the concept of a "fast-slow" economic spectrum to characterize plant nutrient acquisition strategies, which are based on the investment in root attributes (Freschet et al. 2021). In order to acquire competitive advantage for soil nutrients, plants develop efficient morphological structures in their fine roots, such as high specific root length (SRL), specific root area (SRA), low root tissue density (RTD), and low root average diameter (RAD) (Valverde-Barrantes et al. 2017). Plants shape unique rhizosphere microbial communities through these root functional traits, as well as through chemosensory substances released into the rhizosphere (Badri and Vivanco 2009). Although previous studies have shown that the composition and diversity of rhizosphere microbial communities differ among tree species due to differences in physiological traits (Si et al. 2018), the effects of root functional traits on rhizosphere microbial community composition and ecological functions remain uncertain.

The rhizosphere serves as a dynamic interface where plant roots and soil microorganisms engage in intricate interactions, characterized by higher microbial density compared to the bulk soil (Berendsen et al. 2012). Within complex microbial communities, a small number of dominant species are relatively abundant, while a large number of species are rare and have low abundance (Nemergut et al. 2011). The abundant taxa, widely distributed in soils, are considered to be the primary contributors to matter cycling and metabolism (Egidi et al. 2019; Pester et al. 2010). However, the diversity of abundant taxa is often much lower compared to that of rare taxa and only represents a small portion of microbial diversity (Liu et al. 2023). Additional evidence indicates that rare taxa are involved in several nutrient cycle processes and are essential to the health of ecosystems. (Chen et al. 2020; Lawson et al. 2015). Particularly, rare species can greatly facilitate carbon and

nitrogen cycling in rhizosphere soils, underscoring their importance in maintaining soil ecological functions and nutrient cycling (Cui et al. 2023). Understanding the role of microbial sub-communities in rhizosphere effects is crucial for deciphering plant nutrient acquisition strategies.

Microbial community assembly is an important ecological process that is largely regulated by deterministic and stochastic processes (Xiong et al. 2021b; Xun et al. 2019). Deterministic processes, driven by environmental factors and biological relationships, significantly influence microbial community structure (Jiao et al. 2020), while stochastic processes, affected by historical contingencies and ecological drift, make it challenging to discern community assembly patterns (Gao et al. 2020). The properties of microbial communities formed by different assembly processes vary, which may have important implications for community function (Delgado-Baquerizo et al. 2018). The assembly of rare taxa in the rhizosphere is often dominated by deterministic processes, as these taxa are generally more sensitive to environmental conditions. On the other hand, abundant taxa tend to be influenced more by stochastic processes (Jiao and Lu 2020a; Qin et al. 2022; Wan et al. 2021b). It is important to note that these patterns may vary across different ecological environments (Mo et al. 2018; Wan et al. 2021a). Given the critical role of environmental conditions in shaping microbial community assembly processes (Jiao and Lu 2020b), further research is needed to understand the specific assembly mechanisms of abundant and rare taxa within the rhizosphere.

The Northeast region of China, serving as the principal forest area, has seen extensive plantation establishment as a result of national afforestation programs since the 1970s (Chen et al. 2019). Understanding the complex interactions between tree root systems, rhizosphere microbial sub-communities, and rhizosphere effects in plantation forests becomes crucial. This study seeks to shed light on plant-soil interactions by examining the impact of plant root functional traits and microbial community characteristics on rhizosphere effects. To achieve this, we collected fine roots, rhizosphere and bulk soils from four plantation forests, analyzed root traits and soil nutrient content, and investigated bacterial and fungal communities through high-throughput sequencing of 16S rRNA and ITS genes. Our main objective is to investigate the influence of plant root functional traits on properties of the rhizosphere microbial community and to determine whether the diversity and structure of rare microbial taxa differ significantly compared to abundant taxa. Additionally, we aim to explore the potential greater contribution of rare microbial taxa to rhizosphere effects.

Materials and methods

Site description

The study was conducted at the Keshan (48°12' ~ 48°23'N, 125°8' ~ 125°37'E) in Heilongjiang Province, Northeast China. The Keshan is characterized by black soil, and its climate is continental monsoon, with cold and dry winters and warm and rainy summers. The annual average temperature in the area is recorded at 0.9°C, with an annual average precipitation of 501.77mm.

The four plantations, each 25 years old and consisting of a single tree species, were located adjacently on a gentle slope with a gradient ranging from 2-3°. The four plantations were planted with *Populus* sp. (Pop), *Acer saccharum* (Ace), *Pinus sylvestris* var. *mongolica* (Pin), and *Larix gmelinii* (Lar). Afforestation was carried out using a spacing configuration of 2m×3m, with each plantation covering an area of 20m×100m. Among the plantations, the understory vegetation exhibited high diversity, predominantly composed of *Artemisia mongolica*, *Arctium lappa*, *Setaria viridis*, and *Pedicularis grandiflora*.

Sample collection

In September 2022, we selected five trees without obvious diseases and with similar diameter at breast height in each plantation for the experiment. To ensure a representative sample of the forest, these selected trees within the same plantation were spaced more than 20 m apart. We collected rhizosphere and bulk soil samples (20 rhizosphere and 20 bulk soil samples) following the methodology described by Han et al. (2020). Briefly, we identified coarse root (5-10 cm) around the trunk that could be traced back to the trunk and dug soil to a depth of 10–15 cm along with the coarse root. We then picked out several intact fine roots (<2 mm) attached to coarse roots. The selected fine roots were gently shaken, and these soils that did not adhere to the roots were defined as bulk soil, while those that adhered to the roots were defined as rhizosphere soil. Finally, carefully collect both soils and intact fine roots, making sure to obtain at least 100 g of fresh soil sample. The soil samples were later sieved through a 2 mm sieve and divided into two portions for further analysis. One portion was preserved at -20°C to determine soil enzyme activity and conduct

microbial sequencing, while the other portion was air-dried and analyzed for soil physicochemical properties. Additionally, fine roots were also collected for analysis of root functional traits.

Analysis of soil properties and calculation of rhizosphere effects

Soil organic carbon (SOC) was determined using TOC/TN analyzer (Multi-N/C, Germany). The dissolved organic carbon (DOC) was extracted with water and determined by TOC/TN analyzer. Soil total nitrogen (TN), total phosphorus (TP), alkali-hydrolyzable nitrogen (AN) and available phosphorus (AP) were determined according to Bao (2000).

The rhizosphere effect was employed to quantify the impact of root and rhizosphere processes in comparison to bulk soil processes. It is measured as the difference in the response variable between the rhizosphere and bulk soil, calculated as (rhizosphere soil content - bulk soil content)/bulk soil content.

Root functional traits and soil enzyme activity analysis

The fine roots were scanned using a flatbed scanner set at a resolution of 300 dpi. Five fine roots per tree were determined as replicates. We analyzed the total length, surface area, volume, diameter, and volume of the fine roots using WinRHIZO software (Pro2004b). To determine the dry mass of the fine roots, we dried them and then calculated the specific root length (SRL), specific root surface area (SRA), and root tissue density (RTD).

We analyzed four hydrolytic enzymes, specifically β -1,4-glucosidase (BG), cellobiohydrolase (CBH), β -N-acetylglucosaminidase (NAG), and acid phosphatase (ACP), in soil samples using the method outlined in Xu et al. (2017). We measured the activities of BG, CBH, NAG, and ACP by employing fluorescent substrates containing 4-methylumbelliferone. Enzyme activity was quantified in units of $\text{nmol h}^{-1} \text{g}^{-1}$ of dry soil.

DNA extraction and illumina sequencing

DNA extracted from 0.25 g of soil samples using PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA). The purity and concentration of DNA was assayed using Thermo NanoDrop One (Thermo Fisher Scientific, MA, USA). The 16S rRNA and ITS genes of different regions were amplified using specific primers with 12bp barcode. Bacteria were amplified using primer sets 515F (5'-GTGCCAGCMGCCG CGGTAA-3') and 806R (5' - GGACTACHVGGGGTWTCTAAT-3') to amplify the V4 region of the 16S rRNA gene. Fungi amplified the internal transcribed spacer (ITS) using primer sets ITS1 (5'-CTT GGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3'). Amplification reactions and thermal cycling using a BioRad S1000 (Bio-Rad Laboratory, CA, USA): Initial denaturation at 94°C for 5 min, then 30 cycles of 30 s denaturation at 94°C, annealing at 52°C for 30 s, extension at 72°C for 30 s, and finally final elongation at 72°C for 10 min. The length and concentration of PCR products were detected by 1% agarose gel electrophoresis. The PCR products were then mixed at equilibrium ratios according to the Gene Tools analysis software (Version 4.03.05.0, SynGene) and the mixed PCR products were purified with the E.Z.N. A. Gel Extraction Kit (Omega, USA).

The PE250 sequencing was performed by Illumina Nova6000 platform and 250 bp paired-end reads were generated. After removing the adaptor and primer sequences, non-compliant Tags were filtered using usearch -fastq_mergepairs (V10, <http://www.drive5.com/usearch/>). The quality of the raw data was finally controlled using fastp (version 0.14.1, <https://github.com/OpenGene/fastp>) via a sliding window (-W 4 -M 20) to obtain Clean Tags.

Microbial co-occurrence network construction

Co-occurrence networks were constructed to visualize the relationships between microbial communities. For network construction, a minimum of 10 concurrent Operational Taxonomic Units (OTUs) were retained from the 20 samples. Robust correlations, with absolute values greater than 0.6 and FDR-corrected p values less than 0.05 based on Spearman's correlations, were utilized. The "igraph" R package was employed to construct the microbial co-occurrence networks, which were subsequently visualized using Gephi (<http://gephi.github.io/>).

The identification of potential keystone species is based on their within-connectivity (Z_i) and

among-module connectivity (P_i) in the network. Nodes in the network are divided into four categories: network hubs ($Z_i > 2.5$ and $P_i > 0.62$), module hubs ($Z_i > 2.5$ and $P_i < 0.62$), connectors ($Z_i < 2.5$ and $P_i > 0.62$), and peripheral nodes ($Z_i < 2.5$ and $P_i < 0.62$). Network hubs, module hubs, and connectors are defined as keystone species (Deng et al. 2012).

Data analyses

Abundant and rare taxa were determined based on the relative abundance of OTUs. Specifically, OTUs with a relative abundance greater than 1% in all samples were classified as always abundant taxa (AAT). OTUs with a relative abundance greater than 0.01% in all samples and greater than 1% in some samples were categorized as conditionally abundant taxa (CAT). OTUs with a relative abundance less than 0.01% in all samples were designated as always rare taxa (ART). OTUs with a relative abundance less than 1% in all samples and less than 0.01% in some samples were identified as conditionally rare taxa (CRT). OTUs with a relative abundance less than 1% in all samples and greater than 0.01% in all samples were classified as moderate taxa (MT). OTUs with a relative abundance greater than 1% in some samples and less than 0.01% in others were labeled as conditionally rare and abundant taxa (CRAT). AAT and CAT were considered abundant taxa, MT and CRAT were considered intermediate taxa, and ART and CRT were considered rare taxa (Wang et al. 2023).

Normalized stochasticity ratio (NST) based on Jaccard distances calculations was applied to assess the assembly of rhizosphere microbial communities (Ning et al. 2019). Specifically, $NST > 50\%$ and $< 50\%$ indicated that community assembly was dominated by stochastic and deterministic processes, respectively. The niche breadth of the bacterial and fungal sub-communities was determined using the "spaa" R package.

Statistical analyses and data visualization were conducted using R software (version 4.3.1). One-way analysis of variance (ANOVA) and Tukey test was used to compare differences in rhizosphere effects (soil nutrients and enzyme activities) and root functional traits among different plantations. Prior to performing ANOVA, a normality test was employed on the dataset. If the assumption of normality was violated, a log-transformation was applied to improve the normality of the residuals. The microbial alpha-diversity (Shannon and Pielou indices) and community

composition (principal co-ordinates analysis) were analyzed using the "vegan" R package. Differences in microbial community composition among plantation forests were determined using analysis of variance on distance matrices (Adonis) based on the Bray-Curtis distance measure. The relationships between microbial communities (abundance taxa and rare taxa), rhizosphere effects, root functional traits, and soil properties were assessed using the mantel test from the "linkET" R package. Additionally, Pearson correlation analysis from the "psych" R package was employed to examine the relationship between microbial diversity (abundance taxa and rare taxa), community composition, rhizosphere effects, root functional traits, and soil properties. To investigate the relationship between keystone species and rhizosphere effects, we categorized keystone species to the phylum level and used the mantel test to link keystone taxa to rhizosphere effects. Since there were only five key species for fungi, we analyzed them as a whole. To identify key influences on rhizosphere effects, random forest models were developed using the "randomForest" R package. Each predictor was evaluated using the "rfPermute" R package, and the percentage increase in mean square error (MSE) was used to characterize the predictors. Partial least squares path modeling (PLS-PM) was performed using the "plspm" R package to uncover the potential pathways through which root functional traits and microbial communities affect rhizosphere effects.

Result

Rhizosphere effects on soil nutrients

The rhizosphere effects of different tree species on soil nutrients varied significantly, except for soil organic carbon (Fig. 1). The rhizosphere effects on soil nitrogen TN and AN for Pop were higher than other species. Ace demonstrated a stronger rhizosphere effect on DOC than other species. Pin demonstrated significantly greater rhizosphere effects on TP compared to Pop. Moreover, the rhizosphere effects on AP for Pop were higher than for Lar.

Rhizosphere microbial diversity and community composition

A total of 16669 bacterial OTUs were obtained from 5001602 raw sequences (Table. S3). The OTUs for the abundant, intermediate and rare taxa were 25, 195 and 16449, respectively. A total of 1905

233 fungal OTUs were obtained from 3440889 original sequences (Table. S3). the OTUs for abundant,
234 intermediate and rare taxa were 24, 62 and 1819, respectively.

235 There was variation in the diversity of bacteria and fungi among tree species, but this difference
236 was only significant for rare taxa (Fig. 2). Specifically, the Shannon index of rare bacterial taxa was
237 significantly higher in the rhizosphere soils of Pop compared to Lar. Analogous results were
238 observed for the Pielou index. The Shannon index of fungal rare taxa was as well significantly
239 higher in the rhizosphere of Pin compared to Lar. The composition of bacteria and fungi in the
240 rhizosphere soil differed significantly between tree species (Fig. 3) . Rare bacterial taxa varied
241 more noticeably among tree species compared to abundant taxa.

242 According to the NST analysis based on the Jaccard distance, in the bacterial community, the
243 NST value of AT was below 50%, while the NST value of ALL and RT was above 50% (Fig. 4).
244 This indicates that the assembly of AT is dominated by deterministic processes, whereas the
245 assembly of ALL and RT is dominated by stochastic processes. The assembly processes of AT and
246 RT in fungal communities are similar to those of bacteria, but determinism overwhelms randomness
247 in the assembly of fungal ALL. Moreover, the habitat niche breadth of bacterial communities was
248 higher than that of fungi. Both bacterial and fungal abundant taxa had higher niche breadth than rare
249 taxa.

251 Relationship between microorganisms and rhizosphere effect

252 The Mantel test results demonstrated that the rhizosphere effects on soil TN, DOC, AN and AP were
253 correlated with rhizosphere microbial community (Fig. S1). Specifically, the impact of rhizosphere
254 effect on TN was associated with bacteria rare taxa, as well as fungi abundant and rare taxa.
255 Rhizosphere effect on DOC were linked to bacteria rare taxa and fungi rare taxa. Rhizosphere effect
256 on AN were related to fungal abundance and rare taxa. Rhizosphere effect on AP were associated
257 with abundant bacterial taxa and rare fungal taxa. Additionally, we observed a significant and
258 positive correlation between rhizosphere microbial diversity and rhizosphere effects on TN, AN,
259 and TP (Fig. 5). The community characteristics of rare species exhibited stronger correlations with
260 rhizosphere effects on soil TN and AN.

Microbial co-occurrence network and keystone species

Two co-occurrence networks of bacteria and fungi were constructed based on the correlation of OTUs (Fig. 6). Bacterial networks exhibit a higher number of nodes and edges compared to fungal networks (Table. S4). The main components of the network species nodes were rare taxa, accounting for 93.92% of the bacterial network and 72.13% of the fungal network, respectively. Furthermore, the relationships between the nodes in both bacterial and fungal networks were predominantly positive (Fig. 6).

A total of 344 keystone species were identified in the bacterial community, with 3.68% belonging to abundant taxa and 96.36% to rare taxa (Table. S5). Among the fungi, all five key species belonged to rare taxa. Moreover, our Mantel test revealed that the majority of key taxa of rhizosphere microorganisms were correlated with the rhizosphere effect on DOC (Table. S6).

Factors affecting rhizosphere effects

The results from the Random Forest analysis indicate that the sub-community characteristics of microbes and the rhizosphere effects on enzyme activities can account for the variation in rhizosphere effects on soil TN, TP, DOC and AN (Fig.S2). Specifically, these factors explained 52.6%, 12.59%, 25.29%, and 60.53% of the rhizosphere effects on TN, TP, DOC, and AN, respectively. However, they were unable to explain the rhizosphere effects on SOC and AP.

To identify the pathways mediating the rhizosphere effects of TN and AN, we performed a PLS-PM analysis (Fig. 7). The PLS-PM model explained 55% of the total variance in the rhizosphere effects of TN and AN. Specifically, tree species had a significant effect ($P < 0.001$) on root functional traits, accounting for 65% of the total variance in root functional traits. Root functional traits indirectly influenced the rhizosphere effects of TN and AN through rare taxa of bacteria and fungi. Rare taxa of bacteria had the greatest influence on the rhizosphere effects of TN and AN, with a direct effect of up to 0.74.

Discussion

Regulation of root functional traits on the microbial communities in the rhizosphere

The rhizosphere microbial communities play a crucial role in driving various ecological functions (Xiong et al. 2021a; Yue et al. 2023). Our findings indicate a significant clustering pattern in the bacterial and fungal communities among tree species (Fig. 3). This observation demonstrates the pronounced selectivity of the rhizosphere in shaping soil microbial community composition, which may account for differences in rhizosphere effects among tree species. Notably, the composition of rare bacterial taxa shows a more pronounced differentiation among tree species compared to abundant taxa (Fig. 3). This phenomenon may be attributed to the regulation of plant root functional traits. Our study revealed a notable correlation between the diversity and community composition of rare bacterial taxa and root traits, including SRL, SRA and RTD (Fig. 5). These results findings suggest that root functional traits may regulate the diversity and composition of rare bacterial taxa. In contrast, abundant bacterial taxa, due to their higher growth rates and broader adaptability (Campbell et al. 2011), are less influenced by root traits.

In the context of the root economics spectrum, high SRL and SRA are considered to be acquisitive traits, while high root tissue density and root diameter are considered conservative traits (Sun et al. 2021). Our findings demonstrate that high SRL and SRA enhance the impact of the rhizosphere on carbon and phosphorus acquisition enzymes, whereas high root tissue density reduces this effect (Fig. 5). These results support the classification of SRL and SRA as belonging to the acquisitive/fast end of the fast-slow/acquisitive-conservative axis, whereas root tissue density falls under the conservative/slow end of the axis (Han et al. 2020; Sun et al. 2021). However, we observed a positive correlation between RAD and rhizosphere effects on NAG (Fig. 5). This finding seems to contradict the theory of the root economics spectrum. This finding can be explained by the higher resistance to degradation of litter under coniferous trees in plantation forests (Wu et al. 2013). To adapt to this challenge, plants may need larger root diameters to optimize nutrient uptake (Li et al. 2019), especially for soil nitrogen. This also potentially account for the disparity in root diameters observed between Lar and Pin compared to Ace and Pop (Table. S2). These results suggest that root functional traits regulate nutrient acquisition strategies of plants and emphasize the potential influence of root functional traits on rhizosphere rare taxa. The association between root diameter and rhizosphere effects on NAG challenges the theory of root economics spectrum. Subsequent studies on root characteristics and nutrient acquisition strategies should consider the distinctions

between planted and natural forests and investigate the underlying causes for these variances.

Co-occurrence networks and assembly processes of abundant and rare taxa in rhizosphere

In our study, we observed that the majority of the interactions among nodes in the fungal and bacterial co-occurrence network were positive (Fig. 6), aligning with findings from research on microbial communities in wheat rhizosphere soil (Fan et al. 2018). This finding indicates a prevalence of cooperative interactions among microbial species in rhizosphere soil. The rhizosphere is a soil environment abundant in resources, where approximately 17% of the carbon fixed by photosynthesis is allocated through root exudates (Nehls et al. 2016). These additional resource inputs may decrease competition among microbial species, resulting in fewer negative correlations within the network (Costello et al. 2012). We found that both the nodes and edges in the co-occurrence network of bacteria were higher than in fungi (Table. S4), implying a greater level of network complexity in bacteria. This could be attributed to the differences in metabolic predictions between these two groups. Compared to fungi, most bacteria are more susceptible to changes in environmental conditions and the abundance of other taxa (Preusser et al. 2019). On the other hand, fungi can degrade complex organic matter in the soil through enzymatic reactions, which reduces their specific dependence on other microbial communities (Pivato et al. 2008). Consequently, fungal species exhibit fewer interconnections.

Keystone species play a crucial role in shaping network structures and are essential for various ecological functions (Banerjee et al. 2018; Han et al. 2022). Our findings revealed that almost all keystone species were part of rare taxa (Table. S5), highlighting the notable impact of rare taxa on network structures and stability. As key species, rare taxa govern microbial growth and interactions, thereby impacting ecosystem functions (Wang et al. 2023). After categorizing bacterial keystone species at the phylum level, it was observed that the majority of key taxa in the study were linked to the rhizosphere effect on dissolved organic carbon (Table. S6). The preservation and regulation of soil microbial community stability and functionality are closely associated with dissolved organic carbon (Bongiorno et al. 2019; Sokol and Bradford 2019). The microbial community's interactions with dissolved organic carbon play a crucial role in regulating nutrient cycling, decomposing organic matter, providing plant nutrition, and resisting harmful biological invasions (Erktan et al.

2018; Ling et al. 2022). This finding enhances our understanding of how rhizosphere microbial communities are assembled and formed.

Based on a normalized stochasticity ratio analysis, we determined that stochastic processes had a stronger effect on rhizosphere bacterial communities than on fungi (Fig. 4). Quantification of habitat niche breadths at the community level revealed that bacteria have wider ecological niche breadths than fungi (Fig. 4). Narrower niche breadths make fungi more sensitive to environmental stresses and plant-soil interactions, and thus fungal communities are dominated by deterministic processes (Ku et al. 2023). In addition, we found that both bacterial and fungal abundant taxa had wider habitat niche breadths than rare taxa (Fig. 4), suggesting that rhizosphere abundant taxa are more broadly adapted and less influenced by the root system. Although the abundant taxon has a wider habitat niche breadth, its community assembly processes are more influenced by deterministic processes. Similar results were found in subtropical gulfs (Mo et al. 2018) and oil-contaminated soils (Hou et al. 2020), which may be partly attributed to spatial and temporal scale dependence and habitat specificity (Liu et al. 2020; Wang et al. 2017).

The contribution of microbial sub-communities to rhizosphere effects.

Plants can influence the structure and function of microbial communities through the regulation of rhizosphere microbial community assembly (Pérez-Jaramillo et al. 2016), which could be a key factor in driving changes in rhizosphere effects. We identified more correlations between the diversity and community composition of rare taxa and rhizosphere effects on TN and AN (Fig. 5). The results of the PLS-PM and random forest model also suggest that the properties of rare taxa contributed significantly to the rhizosphere effects on TN and AN (Fig. 7 and Fig. S2). Previous research conducted in acid mine drainage showed that rare taxa were responsible for nitrogen cycling, particularly nitrogen fixation (Hua et al. 2015). Additionally, rare taxa also made significant contributions to soil denitrification (Shu et al. 2018). Therefore, rare taxa play a crucial role in the rhizosphere effects on nitrogen.

However, we found that the characteristics of microbial communities could not explain the rhizosphere on soil organic carbon and available phosphorus (Fig. S2). One possible reason is the presence of high organic carbon content in the black soil of northeastern China, which leads to a

limited influence of microbial communities on soil organic carbon levels (Liu et al. 2015). On the other hand, the results of the Mantle test indicated a significant correlation between the effects of the rhizosphere on available phosphorus and both abundant bacterial and rare fungi taxa (Fig. S1). This finding suggests that while microbial community diversity and composition cannot fully account for the impact of the rhizosphere on available phosphorus, it does not imply that microbial communities and rhizosphere effects on phosphorus availability are unrelated. In an agricultural field experiment, it was observed that rare taxa make a substantial contribution to phosphorus mineralization. (Cui et al. 2023). In summary, Trees can induce changes in rhizosphere microbial communities through fine root traits, which ultimately produce rhizosphere effects of soil nitrogen.

Conclusion

In plantation forests, the regulation of rhizosphere microbial communities and ecosystem functions by tree roots is a complex process. The study revealed significant differences in the rhizosphere effects of soil nutrients across tree species. Root functional traits were identified as key regulators of rhizosphere microbial diversity and community composition. Moreover, the study found a strong association between rhizosphere microbial key taxa and the effects of dissolved organic carbon in the rhizosphere. Additionally, the properties of rare rhizosphere microbial taxa communities emerged as crucial factors influencing the rhizosphere effects of nitrogen and alkali-hydrolyzable nitrogen. Overall, these findings provide valuable insights into the intricate mechanisms by which tree roots shape rhizosphere microbial communities and drive ecosystem functions in plantation forests.

Statements & Declarations

Funding

This research was funded by the National Key R&D Program of China (2021YFD1500705), the Fundamental Research Funds for the Central Universities (2572023AW16).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Conceptualization, X. W and L. P.; data curation, X. W and L. P.; formal analysis, X. W. and L. P.; funding acquisition, X. W.; methodology, X. W and L. P; project administration, X. W.; software, L. P. and X. W; supervision, X. W; visualization, X. W.; writing original draft, X. W and L. P; writing review and editing, X. W. All authors have read and agreed to the published version of the manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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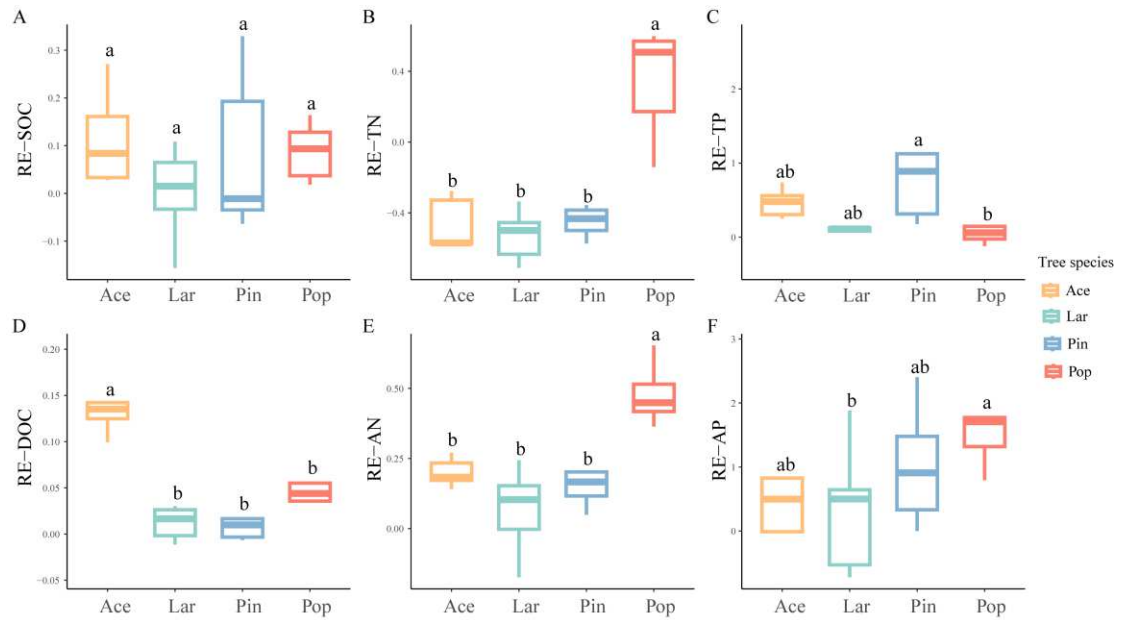


Fig.1 Interspecific variation in the rhizosphere effects on soil nutrient properties in four tree plantations. Box plot colors are used to differentiate between tree species. Different lowercase letters indicate significant differences ($p < 0.05$). Ace: *Acer saccharum*, Lar: *Larix gmelinii*, Pin: *Pinus sylvestris* var. *mongolica* and Pop: *Populus* sp. RE- indicates the rhizosphere effects. SOC: soil organic carbon, TN: total nitrogen, TP: total phosphorus, DOC: dissolved organic carbon, AN: alkali-hydrolyzable nitrogen and AP: available phosphorus.

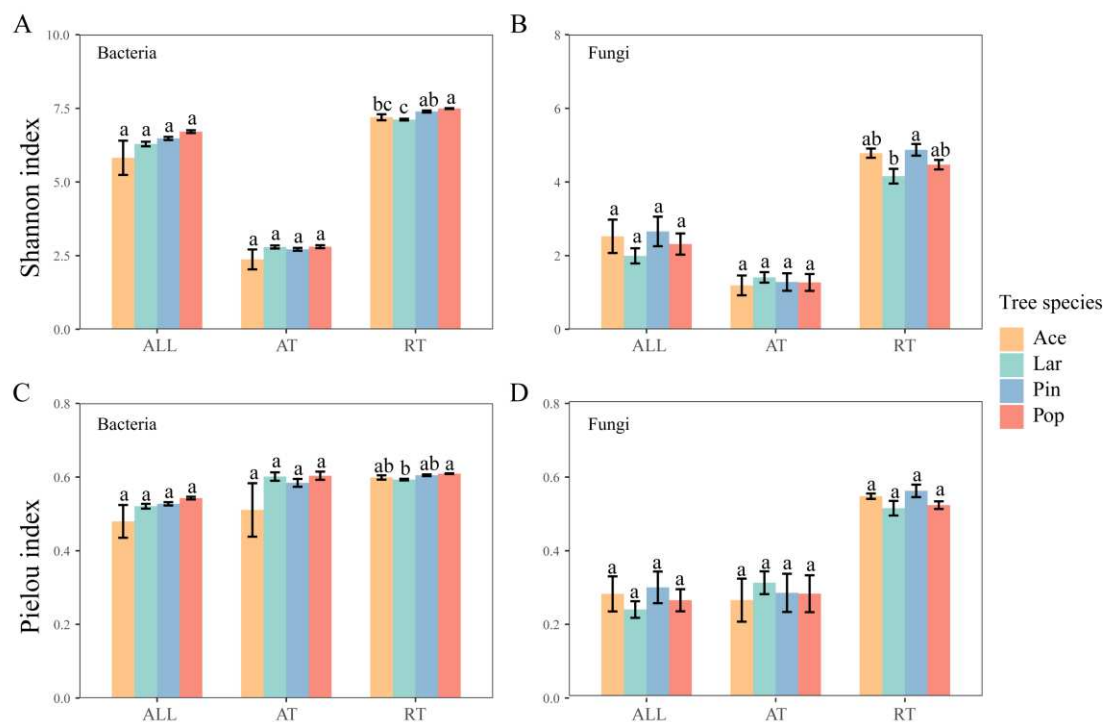


Fig. 2 Diversity indexes for all, abundant and rare taxa of (A and C) bacteria and (B and D) fungi. Lowercase letters indicate significant differences between tree species ($p < 0.05$). (A) Bacterial Shannon index; (B) Fungal Shannon index; (C) Bacterial Pielou Index.; (D) Fungal Pielou Index. Ace: *Acer saccharum*, Lar: *Larix gmelinii*, Pin: *Pinus sylvestris* var. *mongolica* and Pop: *Populus* sp. ALL: all taxa; AT: abundant taxa and RT: rare taxa.

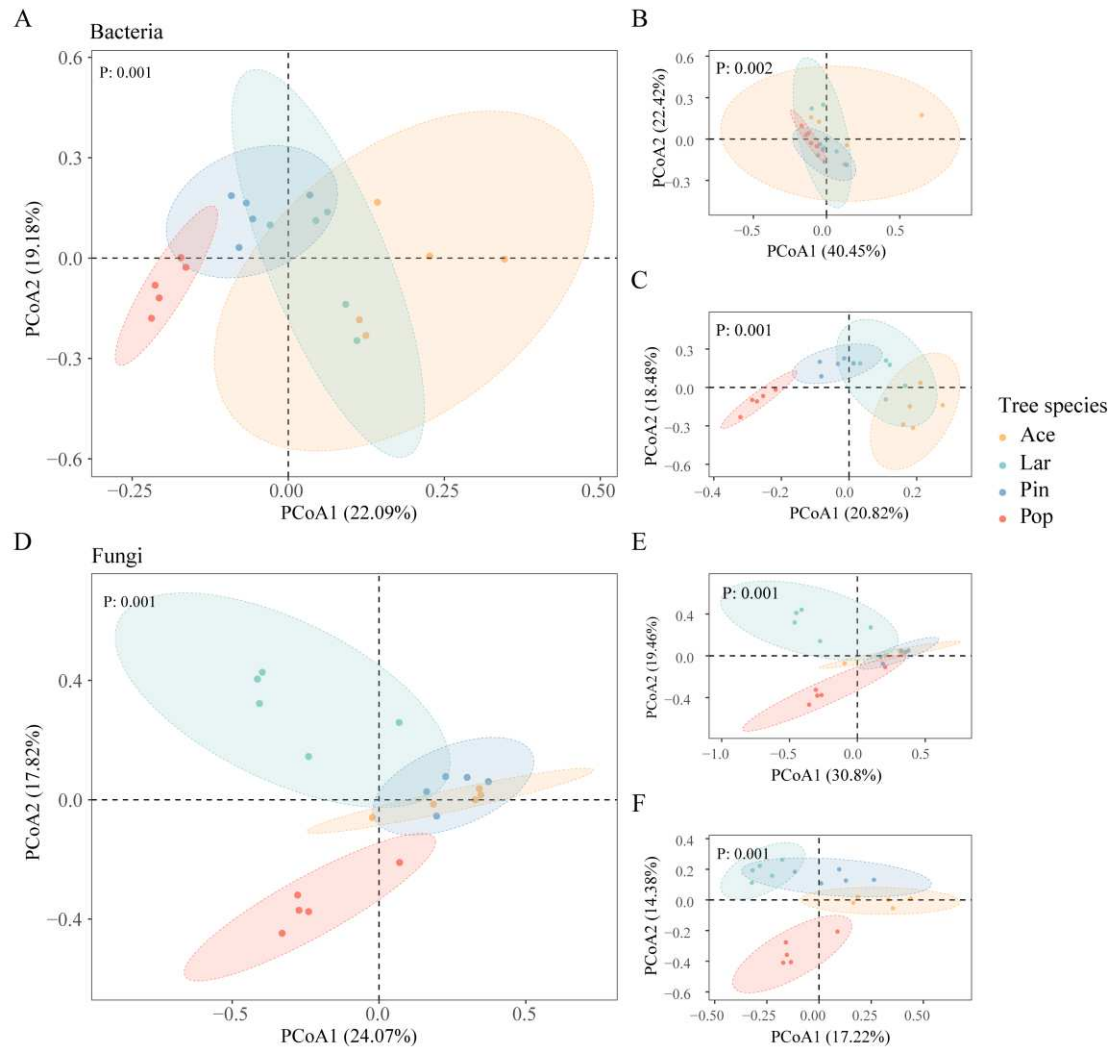


Fig. 3 Principal co-ordinates analysis (PCoA) based on Bray–Curtis distances, showing the compositions of rhizosphere soil microbes from four plantations. (A) bacterial all taxa, (B) bacterial abundant taxa, (C) bacterial rare taxa, (E) fungal all taxa, (F) fungal abundant taxa, (G) fungal rare taxa. Ace: *Acer saccharum*, Lar: *Larix gmelinii*, Pin: *Pinus sylvestris* var. *mongolica* and Pop: *Populus* sp.

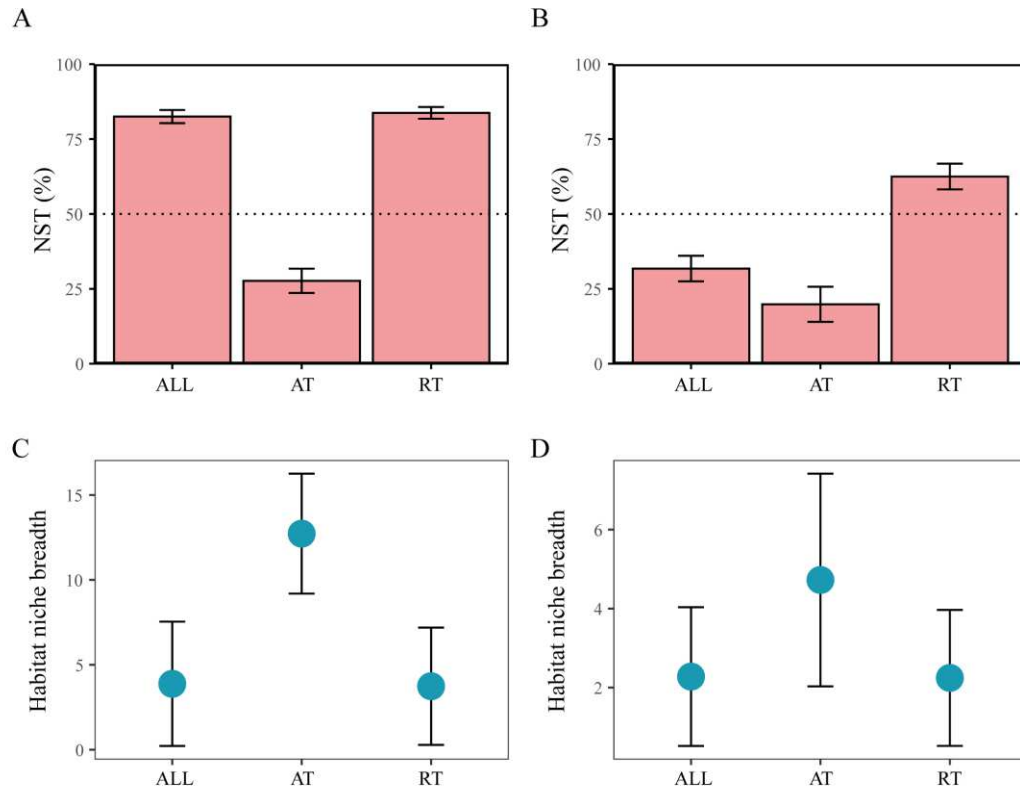


Fig. 4 Patterns of community assembly processes in rhizosphere (A) bacteria and (B) fungi as measured by the normalized stochasticity ratio (NST) index calculated based on Jaccard distances. Habitat niche breadth of rhizosphere bacteria (C) and fungi (D) was analyzed at the community level. ALL: all taxa; AT: abundant taxa and RT: rare taxa.

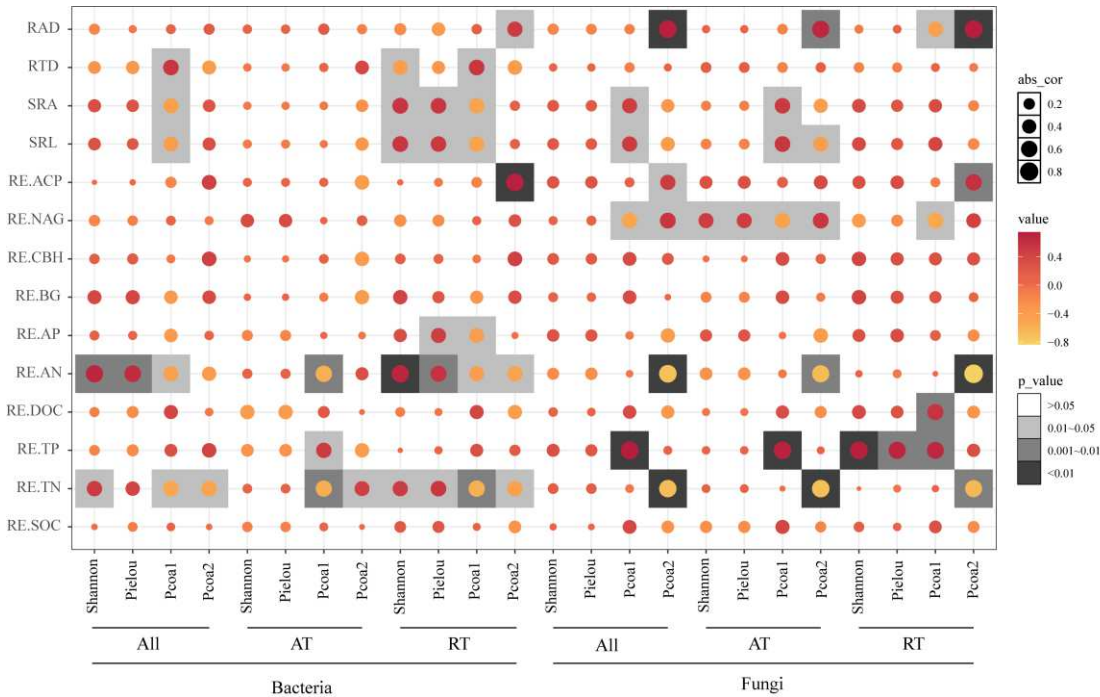


Fig. 5 The relationships between the diversity and community composition of rhizosphere microorganisms and rhizosphere effects and functional root traits. ALL: all taxa, AT: abundant taxa and RT: rare taxa. RE- Indicates the rhizosphere effect. SOC: soil organic carbon, TN: total nitrogen, TP: total phosphorus, DOC: dissolved organic carbon, AN: alkali-hydrolyzable nitrogen, AP: available phosphorus, BG: β -1,4-glucosidase, CBH: cellobiohydrolase, NAG: β -N-acetylglucosaminidase and ACP: acid phosphatase. SRL: specific root length, SRA: specific root area, RTD: root tissue density and RAD: root average diameter.

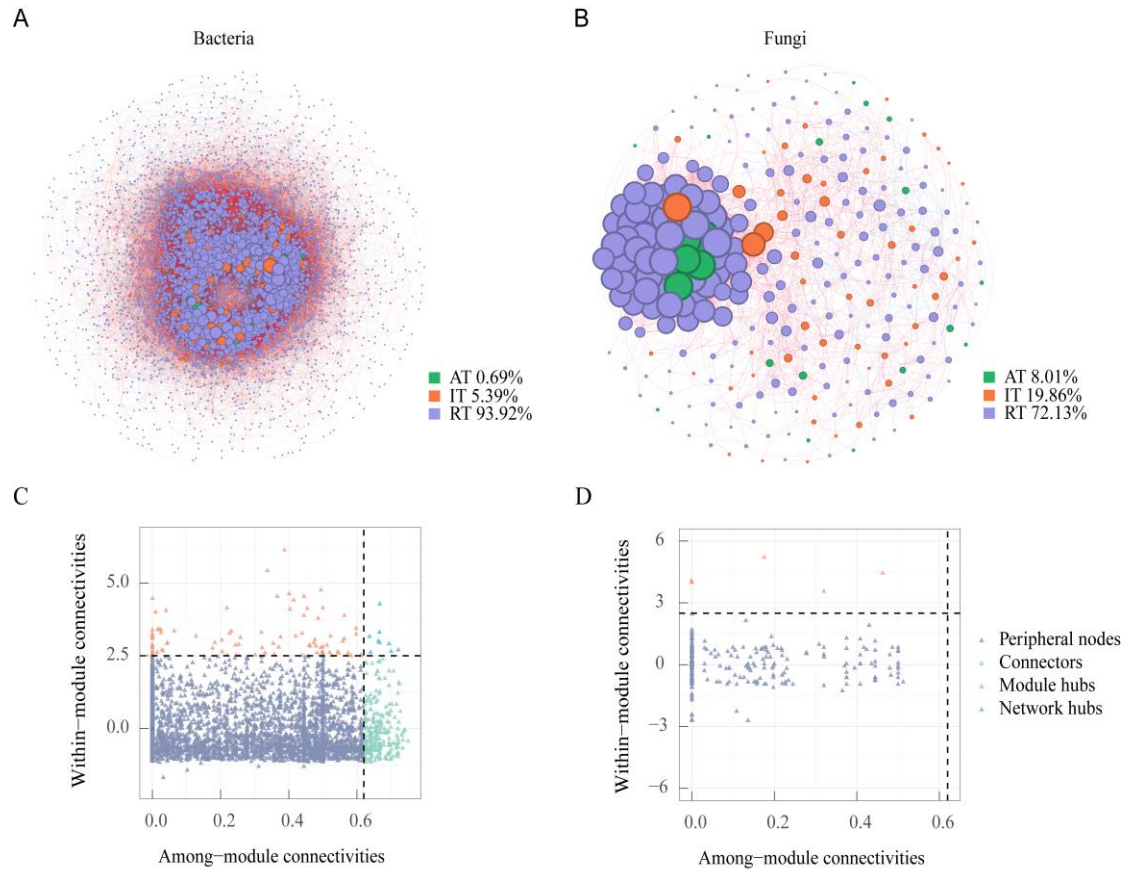
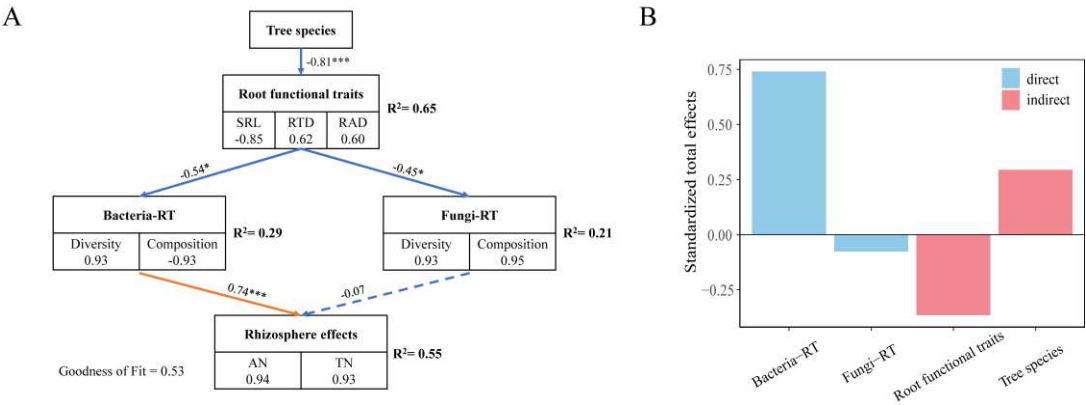


Fig. 6 Co-occurrence networks of bacterial (A) and fungal (B) communities. Identification of key OTUs of bacteria (C) and fungi (D). The size representation of nodes in Co-occurrence networks is proportional to the degree. The red lines between nodes indicate positive interactions and the blue lines indicate negative interactions. AT: abundant taxa, IT: intermediate taxa and RT, rare taxa.

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657 Fig. 7 Partial Least Squares Path Model (PLS-PM) showing the direct and indirect effects of tree
658 species, root functional traits, rare taxa of fungi and rare taxa of bacteria on rhizosphere effects. The
659 indicated values are the path coefficients. The numbers under the factors represent loading. Orange
660 lines indicate positive paths, whereas blue lines indicate negative paths. R^2 values denote the
661 variance of variables accounted for by the model. *, ** and *** indicate significance at $P < 0.05$, P
662 < 0.01 and $P < 0.001$, respectively. SRL: specific root length, TD: root tissue density, RAD: root
663 average diameter, TN: total nitrogen and AN: alkali-hydrolyzable nitrogen. Bacteria-RT: rare taxa
664 of bacteria and Fungi-RT: rare taxa of fungi.

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Supplementary Files

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