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Effects of Plant Fine Root Functional Traits and Soil Nutrients on the Diversity of Rhizosphere Microbial Communities in Tropical Cloud Forests in a Dry Season

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Abstract: The composition and diversity of rhizosphere microbial communities may be due to root–soil–microbial interactions. The fine root functional traits and rhizosphere soil environmental factors of 13 representative plants in the Bawangling tropical cloud forest of Hainan Island were measured, to assess the key factors driving plant rhizosphere microbial communities. Illumina MiSeq sequencing technology was used to sequence the v3-V4 region of the 16SrDNA gene of 13 plant rhizosphere soil bacteria and the ITS1 region of the fungal ITSrDNA gene. Results showed that there were 355 families, 638 genera, and 719 species of rhizosphere soil bacteria as well as 29 families, 31 genera, and 31 species of rhizosphere soil fungi in the tropical cloud forests. The fine root traits, such as root phosphorus content, the specific root length and specific root area, were significantly negatively correlated with the Faith-pd indices of the bacterial community but were not correlated with the diversity of fungi communities. The soil pH was significantly and positively correlated with the Chao1 index, OTUs, Faith-pd and Simpson indices of the bacteria and fungi communities. The soil available phosphorus content was significantly and negatively correlated with the bacteria Simpson and the fungus Faith-pd indices. ABT analysis showed that soil pH and soil available phosphorus were the most important environmental conditions contributing to the rhizosphere bacterial and fungi communities, respectively. Our findings demonstrate that the soil environments had more influence on rhizosphere soil microbial diversity than the fine root functional traits.

Keywords: Hainan island; soil pH; soil available phosphorus; root–soil–microbial interactions



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1. Introduction

The rhizosphere is the soil region directly affected by plant roots and is the place where roots, soil microorganisms, and soil interact [1,2]. Due to the effects of the rhizosphere, there are more microorganisms in rhizosphere soil than in bulk soil [3]. The community structure, abundance, and diversity of the rhizosphere microorganisms are affected by root–soil–microbe interactions [4]. Plant roots and soil are two important components of the root–soil–microbe interface and are the main factors that influence the composition and structure of the rhizosphere microbial community [5,6].

Plant roots continuously secrete various substances to promote plants' absorption of mineral elements and provide the rhizosphere soil microbes with sugars, sugar alcohols, amino acids and phenolics as nutrient and energy supplies [7]. The type and quantity of the root exudates determine the type and quantity of rhizosphere microorganisms and affect the rhizosphere soil microbial community structure and carbon source utilization [8]. That is to say, the plants "actively" select rhizosphere soil microorganisms through root exudates [9].

As the main plant organs for the absorption, storage, and transport of nutrients and water of the plant root system, fine roots (diameter ≤ 2 mm) are crucial for the growth and distribution of plants [10]. They are sensitive to the changes in the soil environment and adapt to the environmental changes by changing their shapes and other characteristics [11]. Studies have found that the functional traits of fine roots are significant for predicting soil microbial groups and functional communities [12,13]. For example, Sweeney et al. (2021) found that temperate grassland plant functional traits, especially root traits, affect the composition of the rhizosphere fungal community and can be used to predict the fungal community. Similarly, Spitzer et al. (2021) found that the spectrum of subarctic tundra meadow plants' fine root economic trait chemical axis is positively correlated with the rhizosphere fungus/bacteria ratio. Although many studies have focused on the relationship between root traits and rhizosphere soil microbes [12,14–20], no studies we are aware of have explored these relationships in a high-altitude tropical forest ecosystem.

Soil is one of the ecosystems with the most abundant microbes on earth and is regarded as a reservoir for rhizosphere microbial communities [21]. The complex properties of soil affect the initial microbial community during the assembly process of the rhizosphere soil microorganisms by directly changing the soil microbial community composition [21]. Furthermore, soil can indirectly change the composition and relative abundance of the rhizosphere soil microbiome by influencing plant physiological activities [22]. For example, Zhao et al. [23] found that the nutrient content of soil is the main factor affecting the soil microbial structure of subtropical mountain forests. Glassman et al. [24] showed that pH and soil nutrient locally drive the assembly of a fungal community; global meta-analyses also indicate that soil physical and chemical properties, especially soil pH, are the dominant factors affecting the characteristics and diversity of soil microbial communities [25–28].

Few studies were conducted to examine the combined effect of rhizosphere soil factors and fine root functional traits on rhizosphere soil microorganisms [21], especially in high-altitude forests (such as tropical cloud forests). The tropical cloud forest is a typical ecosystem sensitive to climate change, and is the most threatened and least studied forest in the world [29]. Compared with low-altitude tropical forests, tropical cloud forests have unique community structures and rich species diversity and are mainly distributed on high-altitude mountain tops or ridges, with frequent occurrence of clouds and fog, low temperatures, strong winds, low tree heights, small tree diameter and frequently water-saturated soil [30].

In this study, our objective was to explore the effects of the rhizosphere environment and fine root functional traits on rhizosphere microbial communities in tropical cloud forest. Our hypotheses are: (1) plant fine root functional traits and rhizosphere soil nutrients together affect the rhizosphere microbial community diversity; (2) the soil abiotic environment plays a predominant determinant role in the assembly of the rhizosphere microbial community.

2. Materials and Methods

2.1. Study Site

The study site was located in the Bawangling area of Hainan Tropical Rainforest National Park (18°50'–19°05'N, 109°05'–109°25'E), with an altitude range of 100–1654 m (Figure 1). This region has a tropical monsoon climate with obvious dry and wet seasons, with a wet season from May to October and the dry season from November to April [31]. The typical type of soil is latosol developed from granite and sandstone as parent materials, which gradually transitions into mountain red soil, yellow soil and meadow soil with an increased altitude [32]. The main vegetation types include lowland rainforest, mountain rainforest and cloud forest [33]. The tropical cloud forest in Bawangling is mainly distributed in the shape of islands on ridges or mountaintops above 1250 m above sea level. There are two forest types in the tropical cloud forest in Bawangling, which are generally categorized as a tropical montane evergreen forest (TMEF) and a tropical dwarf forest (TDF) [32]. They are primary old-growth forests. The dominant species in TMEF include

Cryptocarya chinensis (Hance) Hemsl., *Cyclobalanopsis championii* (Benth.) Oerst., *Ternstroemia gymnanthera* (Wright et Arn.) Sprague, *Exbucklandia tonkinensis* (Lec.) Steen., *Cinnamomum tsoi* Allen. and *Syzygium araiocladum* Merr. et Perry. In TDF, dominant species include *Distylium racemosum* Sieb. and Zucc., *Syzygium buxifolium* Hook. et Arn., *Engelhardtia roxburghiana* Wall., *Symplocos poilanei* Guill., *Rhododendron moulmianense* Hook. f and *C. tsoi* Allen [32]. The species in tropical cloud forest systems are assembled with multiple ecological processes [34]. The tree and shrub species within the tropical cloud forest were non-random trait-based assembled [35].

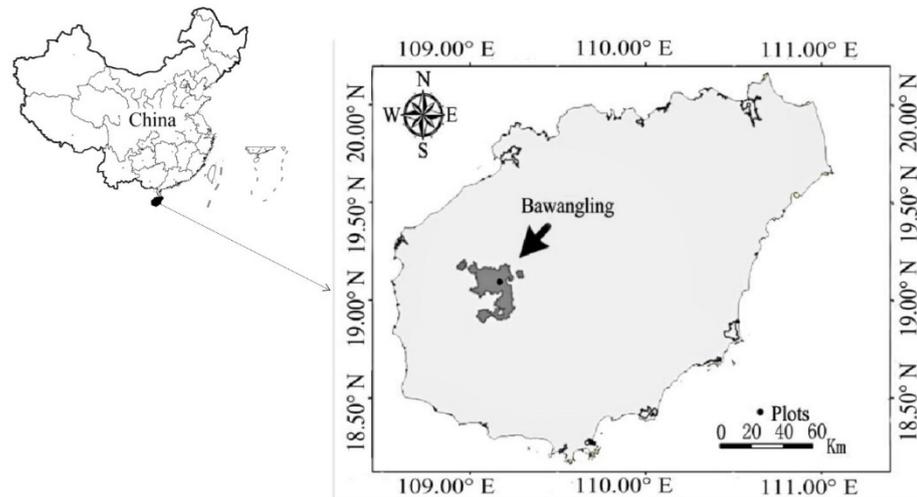


Figure 1. Location map of the study area.

2.2. Samples Collection

The experimental samples were collected in January 2021. According to the early plant species diversity, the data of 21 (20 m × 20 m) plots with diameter at breast height (DBH) larger than 1 cm in Bawangling tropical cloud forest and considered as community dominant species, rare species, phylogenetic relationships of plants, plant classification groups (gymnosperms and angiosperms), and plant life forms (shrub and macrophanerophytes) were obtained. In the present study, we chose 13 representative tree species (Table 1). DBH was measured by DBH ruler at the height of 1.3 m above the ground, and plant height of all individual trees appearing in the study plot was measured using a clinometer.

After clearing debris about 1 m around the target tree trunk (inside the crown), fine roots were collected by the root-tracking method. After digging out the fine roots, large pieces of soil were shaken off and soil within 5 mm on the root surface was collected [36]. For the soil samples, after a soil sample was taken from each of the three different directions of the plant with 10–30 cm depth (there were 3 soil samples of a plant taproot), then the three soil samples were mixed together. Finally, we obtained a mixed soil sample for each individual plant. For the root samples, a root sample was taken from each of the three different directions, and then we obtained three root samples for each individual plant. Altogether, 56 soil samples and 168 root samples were collected. The rhizosphere soil samples were brought to the laboratory for air drying and determination of physical and chemical properties. Furthermore, the soil samples were stored in $-80\text{ }^{\circ}\text{C}$ liquid nitrogen for high-throughput rhizosphere soil microbial sequencing. The fine root samples were put into moisturizing sample bags and were placed in a cryogenic storage box. After the cryogenic storage box was transported back to the laboratory, the root samples were put into refrigerator at $4\text{ }^{\circ}\text{C}$.

Table 1. Plant life forms, categories, stem diameter and height of plant species involved in the study.

Plant Species	Plant Life Forms	Plant Categories	DBH ¹ (cm)	Height ¹ (m)
<i>Michelia mediocris</i> Dandy	Tree	Angiosperm	6.22 ± 5.31	5.76 ± 2.43
<i>Podocarpus neriifolius</i> D. Don	Tree	Gymnosperm	5.62 ± 5.19	4.600 ± 1.39
<i>Syzygium buxifolium</i> Hook. et Arn.	Shrub or small tree	Angiosperm	8.02 ± 3.29	5.560 ± 1.13
<i>Cyclobalanopsis disciformis</i> (Chun et Tsiang) Y. C. Hsu et H. W. Jen	Tree	Angiosperm	7.62 ± 11.45	4.18 ± 2.20
<i>Manglietia fordiana</i> var. <i>hainanensis</i> (Dandy) N. H. Xia	Tree	Angiosperm	1.76 ± 0.53	2.90 ± 1.25
<i>Pinus fenzeliana</i> Hand.-Mzt.	Tree	Gymnosperm	36.24 ± 16.41	12.14 ± 5.21
<i>Castanopsis fabri</i> Hance	Tree	Angiosperm	2.63 ± 1.79	2.50 ± 0.87
<i>Osmanthus didymopetalus</i> P. S. Green	Tree	Angiosperm	2.92 ± 2.56	4.12 ± 1.89
<i>Distylium racemosum</i> Sieb. et Zucc.	Shrub or small tree	Angiosperm	16.46 ± 4.17	9.00 ± 1.00
<i>Allomorpha balansae</i> Cogn.	Shrub	Angiosperm	2.44 ± 1.45	2.75 ± 0.60
<i>Olea dioica</i> Roxb.	Shrub or small tree	Angiosperm	2.17 ± 1.10	2.93 ± 0.66
<i>Syzygium championii</i> (Benth.) Merr. et Perry	Shrub to small tree	Angiosperm	2.54 ± 1.59	3.820 ± 2.27
<i>Melastoma penicillatum</i> Naud.	Shrub	Angiosperm	2.13 ± 0.79	3.00 ± 0.84

¹ The values presented are “mean ± standard error”; DBH: diameter at breast height.

2.3. Selection and Measurement of Fine Root Functional Traits

We selected fine root morphology and chemical traits that reflect plant ecological strategies which are closely related to rhizosphere microorganisms, such as specific root length, root tissue density, specific root area, root carbon content, root nitrogen content and root phosphorus content (Table 2). The values of three root samples from the same plant were averaged and taken for the fine root functional traits of each plant.

Table 2. Fine root functional traits selected and ecological strategies.

	Traits	Abbreviation	Unit	Ecological Strategies
Morphology traits	Specific root length	SRL	cm/g	Resource acquisition.
	Root tissue density	RTD	g/cm ³	Transport, support and defense.
	Specific root area	SRA	cm ² /g	Resource acquisition and defense.
Chemical traits	Root carbon content	RC	g/kg	Microbial carbon source.
	Root nitrogen content	RN	g/kg	Microbial nutrient source.
	Root phosphorus content	RP	g/kg	Microbial nutrient source.

The retrieved roots were put into a 0.15 mm mesh bag and the impurities on the root surface were cleaned with low-temperature deionized water. After cleaning, the collected root samples were scanned by digital scanner (ESPON Chops V700 PHOTO). The WinRHIZO Pro 2011B (Regent Instruments, Canada) root image analysis software was used to analyze the scanned fine root images with diameter <2 mm to obtain the information of fine root length (cm), surface area (cm²) and volume (cm³). The scanned and analyzed fine roots were put into marked envelope bags, and then dried in an oven at 65 °C until constant weight was taken out and weighed by an electronic balance (AR2140, Ohaus, USA) to obtain the dry weight. The specific root length, root tissue density and specific root area were calculated by using the following formula [37,38]:

$$\text{Specific root length (cm/g)} = \text{Root length (cm)} / \text{Dry weight of fine root (g)};$$

$$\text{Root tissue density (g/cm}^3\text{)} = \text{Dry weight of fine root (g)} / \text{Fine root volume (cm}^3\text{)};$$

$$\text{Specific root area (cm}^2\text{/g)} = \text{Fine root surface area (cm}^2\text{)} / \text{Dry weight of fine root (g)}.$$

The carbon content of fine roots was determined by potassium dichromate oxidation-external heating method after being crushed through a 40-mesh sieve [39]. After extracting root nitrogen by semi-trace Kelvin method, automatic flow analyzer (ProxiMA1022/1/1, Allians Scientific Instruments Co., LTD., Paris, France) was used to measure the root nitrogen content. Determination of phosphorus content in fine roots was carried out by molybdenum-antimony resistance colorimetry after concentrated sulfuric acid-perchloric acid cooking [39].

2.4. Determination of Soil Physical and Chemical Properties

The collected fresh soil was dried naturally in the laboratory and then crushed by a grinder after 100 mesh sieve was sent for testing. Soil organic matter content was determined by potassium dichromic oxidation-external heating method, soil total nitrogen was extracted by semi-trace Kelvin method and then measured by automatic flow analyzer (ProxiMA1022/1/1, Allians Scientific Instruments Co., LTD., Paris, France), soil available nitrogen content was determined by alkaline hydrolysis diffusion method [40]. The content of total phosphorus in soil was measured by molybdenum-antimony resistance colorimetric method after soil was boiled with concentrated sulfuric acid-perchloric acid [40]. The content of available phosphorus in soil was also measured by molybdenum-antimony resistance colorimetric method [40]. The pH value of soil was measured by potentiometric method [40].

2.5. Sequencing of Rhizosphere Microorganisms

About 5 g of fresh soil samples (kept on dry ice) of each sample were weighed and sent to the Shenzhen Weishengtai Technology Co., Ltd. for fungal ITS and bacterial 16S high-throughput sequencing. Before sequencing, the total DNA was extracted according to the instructions of the E.Z.N.A.[®] soil kit (Omega Bio-Tek, Norcross, GA, U.S.). The DNA concentration and purity were examined by using the NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, DC, USA), and the DNA extraction quality was tested by 1% agarose gel electrophoresis. After qualitative analysis, the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') were used for polymerase chain reaction (PCR) amplification of the hypervariable region of the ribosomal ITS1; the primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used for PCR amplification of the variable region of ribosomal 16S V3-V4. The PCR products were recovered using 2% agarose gel, then purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), eluted with Tris-HCl, examined by 2% agarose electrophoresis, and quantified using the QuantiFluor[™]-ST (Promega, Madison, WI, USA). Based on the Illumina MiSeq sequencing platform, a PE 2*300 library was constructed using the purified amplified fragments following standard operating procedures.

The original sequence fastq file was imported using the import plug-in (part of the QIIME tools) into a file format that can be processed by QIIME2. Then, the QIIME2 dada2 plug-in was used for quality control, trimming, denoising, splicing, and removal of chimeras, and the final characteristic sequence table was obtained. Then, the QIIME2 feature-classifier plug-in was used to compare the ASV representative sequence to the pre-trained database (version 13.8) with a 99% similarity to obtain the classification information table of the species. After that, the QIIME2 feature-table plug-in was used to remove all contaminating mitochondria and chloroplast sequence.

From the sequencing, a barcode tag sequence representing the source information of the sample was obtained, and a valid sequence was identified. Based on the statistics of the effective sequences of the 56 samples collected, a total of 3,276,455 effective sequences were obtained from the 16S sequencing of the 56 tropical cloud forest plant rhizosphere soil samples, of which the lowest contained 44,197 effective sequences and the highest had 73,658 effective sequences. The ITS method gave a total of 3,802,639 effective sequences,

with the lowest number of effective sequences of 46,633 and the highest effective sequences of 145,148.

2.6. Data Analysis

Species annotation and abundance analysis were performed after splicing and filtering Reads and clustering of the Operational Taxonomic Units (OTUs) (97%). The species composition information of rhizosphere soil microorganisms was obtained. The alpha diversity indices, such as OTUs, Chao1, Faith-pd, Shannon, Simpson index, etc., of the rhizosphere soil bacteria and fungi were calculated. The correlation between environmental factors (including soil environmental factors and fine root functional traits) around the rhizosphere microorganisms, as well as the correlation between soil, fine root functional traits, and rhizosphere soil microbes, were analyzed using the Spearman correlation. The OTU abundance of the microbial community was used as the dependent variable, and the functional traits of soil and fine roots were used as independent variables. The relative influence of the functional traits of soil and fine roots on rhizosphere microbial diversity was analyzed using Aggregated Boosted Tree (ABT). All calculations and plots were conducted through R language (R x64 version 4.0.2); ABT analysis was performed using the R language “dismo” package.

3. Results

3.1. Rhizosphere Microbial Diversity

In the present study, 33 phyla, 90 classes, 204 orders, 355 families, 638 genera, and 719 species of rhizosphere soil bacteria were found. The 3 phyla, 11 classes, 22 orders, 29 families, 31 genera, and 31 species of rhizosphere soil fungi were found in the plants collected from the Bawangling tropical cloud forest. The top three ranking phyla in terms of relative abundance among the 33 bacterial phyla are Proteobacteria (25.811–48.381%), Actinobacteria (14.754–48.243%), and Acidobacteria (7.452–37.415%) (Figure 2). One of the three fungal phyla was not annotated, and the other two phyla are Ascomycota (32.453–98.077%) and Basidiomycota (0.395–15.562%) (Figure 3). The Chao1 index of bacteria was found in the range of 547.111–1247.667, the Shannon index 7.867–9.245, and the Simpson index was in the range of 0.988–0.998098189. Similarly, the Chao1 index of fungi was found in the range of 165–896, the Shannon index 1.815–7.144, and the Simpson index ranged from 0.310 to 0.987.

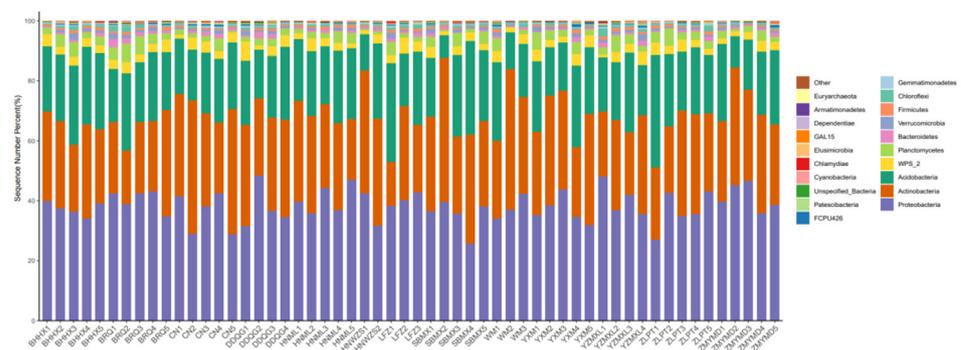


Figure 2. Relative abundance of the first 20 phylum levels of bacterial communities in the samples.

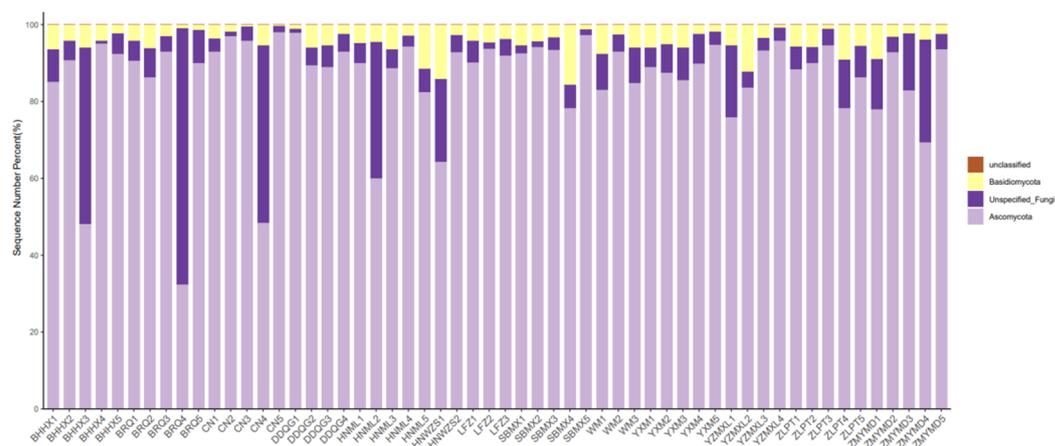


Figure 3. Relative abundance at phylum level of fungal communities in the samples.

3.2. Correlation between Fine Root Functional Traits and Rhizosphere Soil

The nitrogen contents of fine roots were significantly and negatively correlated with soil organic matter ($r = -0.54, p < 0.001$) and total nitrogen content ($r = -0.51, p < 0.001$). The root tissue density was significantly and negatively correlated with soil total phosphorus ($r = -0.35, p < 0.001$). The specific root length (SRL) was significantly and positively correlated with soil available nitrogen ($r = 0.28, p = 0.03$), but significantly negatively correlated with soil pH ($r = -0.32, p = 0.02$). The root area had a significant positive correlation with soil total phosphorus ($r = 0.37, p = 0.005$), and available nitrogen ($r = 0.30, p = 0.02$), and a significant negative correlation with soil pH ($r = -0.33, p = 0.01$) (Figure 4).

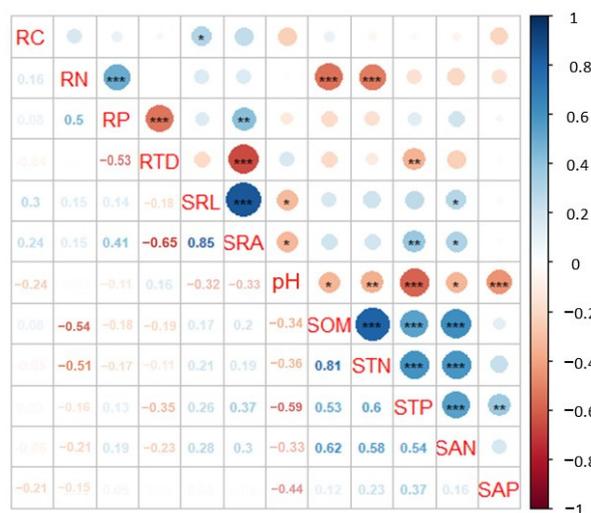


Figure 4. Correlation between fine root functional traits and soil factors (specific root length: SRL; root tissue density: RTD; specific root area: SRA; root carbon content: RC; root nitrogen content: RN; root phosphorus content: RP; soil pH: pH; soil total phosphorus: STP; soil available phosphorus: SAP; soil total nitrogen: STN; soil available nitrogen: SAN; soil organic matter: SOM; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.3. Effects of Fine Root Functional Traits and Rhizosphere Soil on Microbial Diversity

There was a significant negative correlation between the fine root phosphorus content and the OTUs ($r = -0.27, p = 0.04$), Chao1 ($r = -0.28, p = 0.04$), and Faith-pd ($r = -0.27, p = 0.04$) indices of the bacterial community. The specific root length and specific root area of fine roots were significantly and negatively correlated with the Faith-pd index of the bacterial community (specific root length, $r = -0.28, p = 0.04$; specific root area, $r = -0.27, p = 0.04$). The soil pH was significantly and positively correlated with the Chao1 index of

the bacteria ($r = 0.34$, $p = 0.01$), and had a significant positive correlation with the bacteria OTUs ($r = 0.35$, $p = 0.009$), Faith-pd ($r = 0.48$, $p < 0.001$), Shannon ($r = 0.40$, $p = 0.002$), and Simpson indices ($r = 0.37$, $p = 0.004$). The soil pH was significantly and positively correlated with the Shannon index of fungi ($r = 0.31$, $p = 0.02$), and positively correlated with the OTUs ($r = 0.44$, $p = 0.001$), Chao1 ($r = 0.44$, $p = 0.001$), and Faith-pd indices of fungi ($r = 0.48$, $p < 0.001$). The soil available phosphorus content was significantly and negatively correlated with the bacteria Simpson ($r = -0.29$, $p = 0.03$) and the fungus Faith-pd indices ($r = -0.30$, $p = 0.03$) (Table 3).

Table 3. Fine root functional traits and the correlation between rhizosphere soil and soil microbial community diversity.

		RC	RN	RP	RTD	SRL	SRA	pH	SOM	STN	STP	SAN	SAP
Bacteria	OTUs	0.55	0.59	0.04 *	0.23	0.26	0.15	0.009 **	0.85	0.94	0.67	0.16	0.28
	Chao1	0.56	0.57	0.04 *	0.22	0.28	0.16	0.01 *	0.86	0.96	0.66	0.18	0.31
	Faith-pd	0.89	0.33	0.04 *	0.35	0.04 *	0.04 *	0.0002 ***	0.79	0.44	0.43	0.06	0.12
	Shannon	0.34	0.69	0.16	0.32	0.25	0.16	0.002 **	0.85	0.82	0.72	0.31	0.08
	Simpson	0.40	0.60	0.31	0.60	0.22	0.19	0.004 **	0.89	0.93	0.84	0.83	0.03 *
Fungi	OTUs	1.00	0.23	0.47	0.97	0.57	0.56	0.001 **	0.62	0.44	0.37	0.58	0.05
	Chao1	1.00	0.23	0.47	0.97	0.57	0.56	0.001 **	0.62	0.44	0.37	0.58	0.05
	Faith-pd	0.90	0.39	0.47	0.88	0.43	0.47	0.0002 ***	0.40	0.19	0.17	0.99	0.03 *
	Shannon	0.69	0.18	0.85	0.31	0.56	0.33	0.02 *	0.87	0.53	0.79	0.10	0.19
	Simpson	0.89	0.21	0.60	0.25	0.44	0.21	0.06	0.75	0.46	0.86	0.18	0.20

Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; specific root length: SRL; root tissue density: RTD; specific root area: SRA; root carbon content: RC; root nitrogen content: RN; root phosphorus content: RP; soil pH: pH; soil total phosphorus: STP; soil available phosphorus: SAP; soil total nitrogen: STN; soil available nitrogen: SAN; soil organic matter: SOM.

ABT analysis showed that the main environmental factors contributing to the changes in the rhizosphere bacterial community were soil pH (14.1%), fine root carbon content (9.8%), soil organic matter content (9.5%), and soil available phosphorus content (9.3%). The main environmental factors contributing to the diversity of rhizosphere fungi were soil available phosphorus content (10.9%), soil organic matter content (9.9%), soil pH (9.4%), and soil total phosphorus content (8.7%) (Figure 5).

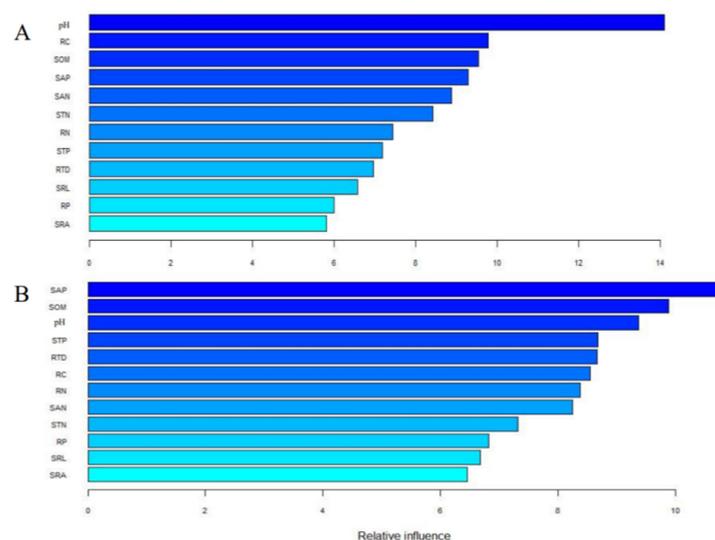


Figure 5. Fine root functional traits and the relative influence of rhizosphere soil on the diversity of rhizosphere microbial community ((A) represents bacteria, (B) represents fungi; specific root length: SRL; root tissue density: RTD; specific root area: SRA; root carbon content: RC; root nitrogen content: RN; root phosphorus content: RP; soil pH: pH; soil total phosphorus: STP; soil available phosphorus: SAP; soil total nitrogen: STN; soil available nitrogen: SAN; soil organic matter: SOM.).

4. Discussion

4.1. Diversity of Rhizosphere Microbial Community

In this study, the Proteobacteria, Actinomycota, and Acidobacteria were the top three dominant phyla in rhizosphere soil bacteria (Figure 2). According to the Global Soil Bacteria Atlas, Proteobacteria and Actinomycota are the two most abundant bacteria in global soils [41]. The Proteobacteria is the largest division among the bacteria, and its members are all Gram-negative bacteria and include multiple metabolic species [42]. Bacteria of the Proteobacteria and Actinomycetes types are the main degraders of organic matter, even complex organic compounds [43]. Another dominant division, Acidobacteria, found in the rhizosphere soil bacteria in this study, belongs to acidophilus, which is consistent with the characteristics of acidic soil in the study area. In tropical regions around the world, forest soils are generally acidic, and their soil microbial compositions are similar. For example, the dominant bacterial phyla of Sarawakian forests in tropical Southeast Asia include Acidobacteria and Proteobacteria [44]. Similarly, the main bacterial phyla of the microbial community of the virgin tropical forest soil in southern Vietnam are Proteobacteria and Acidobacteria [45]. Furthermore, Lan et al. [46] also found that the Acidobacteria are one of the main groups of soil bacterial communities in tropical rainforests when studying the soil microbial community in the tropical rainforest of Hainan Island. Therefore, it may be hypothesized that the composition of rhizosphere bacteria in tropical cloud forest plants might be affected by the combination of the soil bacteria pool and local soil characteristics.

Ascomycota and Basidiomycota are the dominant fungal phyla in the rhizosphere soil of tropical cloud forest plants (Figure 3). Among them, the Ascomycota has the highest abundance and is primarily of the saprophyte type [47]. The main functional microorganisms of saprophytes decompose refractory organic substances and improve the organic matter and soil nutrient content. Saprophytic fungi play a significant role in the soil and atmospheric carbon and nitrogen cycles [48]. Basidiomycota mainly grows in relatively moist soil [49], which is a consequence of the high humidity in the soil environment of tropical cloud forests. Globally, in tropical rainforests with a hot climate and abundant rainfall, the most abundant fungal phyla in the soil include the Ascomycota and Basidiomycota, for example, soil in the tropical rainforest of Queensland, Australia [50]. The soil fungal communities in tropical forests in Puerto Rico are mainly Ascomycota and Basidiomycota [51]. Similarly, Lan et al. [46] found that Ascomycota and Basidiomycota are the major phyla of soil fungi communities in Hainan Island. Therefore, the results of this study indicate that the microbial composition of the rhizosphere soil of high-altitude tropical forests is similar to that of low-altitude tropical forests.

4.2. Impact of the Soil Environment on the Microbial Diversity of the Rhizosphere Soil Relative to the Functional Traits of Fine Roots

In this study, the physical and chemical properties of the rhizosphere soil and the functional characteristics of fine roots significantly influence the rhizosphere soil microbe behavior (Table 3). The studies of Zhao [23], Glassman [24] and other scholars [25–28] mentioned above also showed similar results. Plant roots and rhizosphere soil microorganisms are closely connected, and fine roots and their functional traits affect rhizosphere microorganisms through root exudates and litter quality [13]. The function of specific metabolites in root exudates is indicative of the characteristics of the root economic spectrum, which includes the plant root functional traits. These traits could be used to explain the composition of plant root exudates. Therefore, to a certain extent, root functional traits could also be used to explain the composition and diversity of rhizosphere microorganisms [52]. The results of this study are consistent with the previous studies [12,13,53].

In the tropical cloud forest ecosystem, soil environmental factors have a more significant impact on the rhizosphere soil microbial community than the functional traits of fine roots (Figure 5). Root litter and exudates eventually enter the rhizosphere soil and become important nutrients for rhizosphere microorganisms. Root exudates change the chemical composition of the soil by increasing or decreasing the availability of soil nutrients [54].

Furthermore, the complex physical and chemical properties of the soil cause differences in the initial microbial community during the assembly process of the rhizosphere microbiome, affecting the composition of the rhizosphere microbiome [55]. In addition, the complex interaction between the physical and chemical properties of the soil also affects plant growth and plant physiology, which results in a change in the composition and relative abundance of the rhizosphere microbiome [56,57]. In addition, the mycorrhizal fungi, which by their common mycelial networks interconnect different plants in an ecosystem, further complicate the factors that affect the rhizosphere microbiome. Common mycelial networks (CMNs) can influence plant and microorganism community compositions, induce an efficient nutrient exchange, and improve interplant nutrition and growth through plant-plant facilitation [58]. Mycorrhizas are widespread and abundant, and they are ubiquitous in most temperate and tropical ecosystems [59]. In this study, there are many plant species that have mycorrhizas. For example, *Pinus* is a typical ectomycorrhizal tree genus [60], and *Pinus fenzeliana* Hand.-Mzt belongs to the *Pinus* genus.

4.3. Soil pH Effects on Rhizosphere Soil Microbes in the Soil Environment

Soil pH is the main driving factor for changes in the diversity of bacterial and fungal communities in the rhizosphere soil of tropical cloud forests on Hainan Island (Table 3). ABT analysis shows that soil pH changes the composition of the bacterial and fungal communities most in the tropical cloud forest rhizosphere in Bawangling (Figure 5). Other soil factors, such as SOM, STN, STP, SAN, and SAP, were significantly correlated with soil pH (Figure 4). Changes in soil pH result in changes in the distribution of various nutrient elements in the soil and changes in ion activity, thereby leading to changes in soil fertility [61]. Soil pH also significantly affects the availability of soil nutrients. The solubility of cationic nutrients in strongly alkaline soils decreases, and the sensitivity to loss by leaching or by erosion strongly increases in acidic soils, decreasing the availability of cationic salt nutrients. The availability of anionic nutrients usually shows an opposite trend to that of the cationic nutrients under varying soil pH [62]. Both the cationic and anionic salt nutrients in the soil are essential nutrients for the growth of soil microorganisms and affect the diversity of the microorganisms in the soil. The soil pH is also an important factor influencing cell metabolic activity. Plants select and adapt to rhizosphere bacteria [63]. Plant growth and fine root traits also change accordingly due to the influence of soil pH on soil fertility [64], which also results in changes in the composition and diversity of rhizosphere soil microorganisms. On a large spatial scale, the diversity of soil microbes increases with increasing soil pH [20], and the microbial diversity of acidic soils is usually significantly lower than the neutral soils [65]. The significant positive correlation between soil pH and rhizosphere soil bacterial diversity and fungal diversity found in this study provides new insights into the correlation of the soil pH and the diversity of the rhizosphere soil bacteria in the tropical high-altitude forest ecosystems.

Our results of the Spearman correlation analysis and Aggregated Boosted Tree analysis are also consistent with the meta-analysis results of Zhou et al. [27]. They integrated the results of 1235 global change factors of eight ecosystems including agricultural land, tundra, temperate forests, tropical and subtropical forests, Mediterranean vegetation, grasslands, deserts and wetlands, and concluded that soil pH is the most important factor that can be used to predict the impact of global change factors on microbial alpha diversity [27]. In particular, the results are also consistent with the results of Flores-Rentería et al. [66] and Lan et al. [46]. The composition of soil microorganisms in tropical rainforests is related to soil pH. Tropical cloud forests are high-altitude tropical forests. Therefore, our research provides new insights into the impact of soil factors on rhizosphere microbes. Soil pH in tropical forest communities at varied altitudes is an important factor affecting soil microbial diversity. At the same time, soil temperature is an important riding factor affecting many soil parameters at altitudes >1000 m ASL. Soil acidity at high altitudes is often found due to the low temperatures and accumulation of litter [67,68]. Soil temperature, moreover, affects soil pH mainly by affecting rock weathering rate [69]. Therefore, effects of soil

temperature, an important environmental factor, on rhizosphere microorganisms should also be considered in the future.

4.4. Effects of Soil Available Phosphorus on Rhizosphere Microorganisms

Soil available phosphorus content has a significant impact on the rhizosphere soil bacteria and the rhizosphere soil fungi diversity of tropical cloud forest plants (Table 3). It is also the environmental factor with the greatest impact on the rhizosphere soil fungal community (Figure 5). Phosphorus limitation is more prominent in tropical and subtropical forests, which are characterized by high temperatures, heavy rainfall, and strong weathering and leaching [69]. In tropical cloud forests, the ratio of nitrogen to phosphorus in plant leaves is greater than 17, and plant growth is affected by low soil phosphorus content stress [32]. Among rhizosphere microorganisms, arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) are specific groups of microorganisms that play an important role in the process of soil phosphorus conversion and plant phosphorus absorption [70]. Therefore, soil available phosphorus may be a critical limiting factor for the composition of the rhizosphere soil microbial community in tropical cloud forests. First, the soil available phosphorus content might change the nutritional status of fungi, causing competition between bacteria, soil animals, and also changing fungal groups [71]. In addition, as an important nutrient factor for plant growth, soil available phosphorus changes can result in changes in plant biomass and indirectly affect soil fungi [71]. In addition, soil available phosphorus affects soil microorganisms by influencing other physical and chemical properties of the soil (such as pH) [72]. Cai et al. [73] studied soil microbes in tropical rainforests in Xishuangbanna and also found that soil available phosphorus limits the composition of tropical forest soil microbes.

Our results show that soil pH has a greater impact on the composition of rhizosphere soil bacteria, while soil available phosphorus has a greater impact on the composition of rhizosphere soil fungi. This might be due to the fact that soil fungi can grow in a wide range of pH conditions [74], whereas soil bacteria are more sensitive to soil pH [75]. Another study has shown that fungi solubilize phosphorus better than bacteria [76] and are more sensitive to changes in soil available phosphorus content than bacteria [77,78]. Therefore, fungi are more likely than bacteria to enhance absorption of soil available phosphorus by plant roots by solubilizing phosphorus when there is a demand for available phosphorus in the plant rhizosphere.

5. Conclusions

In this study, we measured the fine root functional traits and rhizosphere soil environmental factors of 13 representative plants in the Bawangling tropical cloud forest of Hainan Island, and assessed the effects of soil conditions and fine root functional traits on rhizosphere microbial communities. We found that both soil conditions and fine root functional traits had important effects on rhizosphere bacteria diversity, but we did not detect the correlations between fine root traits and fungi diversity. The rhizosphere soil environment is more important than fine root functional traits when it comes to affecting rhizosphere soil microbial composition.

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