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Chemical and Microbial Differences of Root and Rhizosphere Soil among Different Provenances of *Fokienia hodginsii*

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Abstract: Aims: *Fokienia hodginsii* is a threatened conifer tree species, known as the dominant nursery-grown species capable of colonizing the challenging woodland environments in southern China due to its strong root penetrating ability. The ecological phenotype of *Fokienia hodginsii* is not well documented during its breeding process, which limits the potential planting area and its ecological function. This study aims to understand how *Fokienia hodginsii* associates with microbes to conduct its key ecological function and provide a theoretical basis for further improving the forest nursery management of *Fokienia hodginsii*. Methods: This study explored the ecological traits of 11 main *Fokienia hodginsii* provenances in a homogeneous garden experiment by analyzing their nutrient utilization strategies and associated microbial features in the rhizosphere soil and roots. Results: The study found that the paramount difference in the rhizosphere soil among provenances is in Ca and Fe content. Some microbial communities, namely Crenarchaeota, Verrucomicrobiota, and Desulfobacterota, were positively correlated with the amounts of the soil nutrient elements, whereas Abditibacteriota and Dependentiae were negatively correlated. The abundance of N- and Fe-related bacteria in the Fu Jian Chang Ting (FJCT) provenance was significantly higher than that in other provenances, while the C-, P-, K-, and Mg-related fungal communities, respectively, had higher abundances in the FJCT, Fu Jian Long Yan (FJLY), Fu Jian Gu Tian (FJGT), and Fu Jian Xian You (FJXY) provenances than the others. The impacts of the Gui Zhou Li Ping (GZLP), Hu Nan Dao Xian (HNDX), Jiang Xi Shang Yao (JXSY), and Guang Dong Shi Xing (GDSX) provenances on the rhizosphere soil are similar, but the differences in nutrient utilization arise from the plant itself. Conversely, the root nutrient contents of the FJCT, Fu Jian You Xi (FJYX), Fu Jian An Xi (FJAX), FJLY, Fu Jian De Hua (FJDH), FJGT, and FJXY provenances are highly correlated with soil nutrient features. Conclusions: For the native provenances, their economic traits are better than the exotic provenances. The native provenances are more sensitive to local soil conditions, so they should benefit more from human interventions, rendering them more suitable for artificial cultivation. The growth of the exotic provenances is less affected by the soil environment, making them better suited for the ecological transformation of forest stands and soil improvement.

Keywords: *Fokienia hodginsii*; genetic background; microbiome; nutrient elements; rhizosphere soil; root

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1. Introduction

Fokienia hodginsii is listed as a vulnerable tree species by the International Union for Conservation of Nature. This threatened species is an ideal landscaping tree because its branchlets are arranged in one plane to form a canopy of tripinnate foliage [1]. Further, *F. hodginsii* has better wood physical properties than the main tree species, Chinese Fir (Cunninghamia lanceolata), planted widely across southern China [2]. The root penetrating ability of *F. hodginsii* is stronger than that of any of the nursery-grown tree species in southern China, including Pinus massoniana, and there are high amounts of N, P, and K nutrients returning in the litterfall of *F. hodginsii* plantations [3]. When compared with P. massoniana, *F. hodginsii* is superior at improving the physical and chemical properties of soil, forest volume, and soil fertility [4–6]. These traits make *F. hodginsi* an excellent substitute for the predominant pioneer species in China, *P. massoniana*, which is now suffering heavily from the pine wilt disease pandemics that poses a serious threat to ecological, biosecurity, and economic development [7–10].

Over 1/2 of the mountainous area in southern China is covered in harsh woodland vegetation, a habitat type that is highly suitable for planting with *F. hodginsii* given its stronger soil improvement ability than other cultivated tree species in southern China. It was recently documented that both human disturbance [11] and global warming [12] are changing the present status of *F. hodginsii* by hastening the degradation and fragmentation of its native habitat, which impairs regeneration of *F. hodginsii* stands in natural forest sites [13]. Thus, establishing seed orchards is imperative for the protection as well as application of *F. hodginsii* [14]. Since the 1970s, seed orchards have been built in many places in Fujian Province, China [15], and the Nanjing State-owned forest farm is the largest one, having collected over 340 varieties from six main distribution provinces (i.e., Hunan, Guangdong, Zhejiang, Guizhou, Jiangxi, and Fujian). Based on 40-year-long field data on plant height, ground diameter, seed yield, and survival rate, 11 high-quality provenances from different geographical regions are distinguishable [16–18]. However, except for its economic traits, other phenotypic aspects of the *F. hodginsii* provenances are not yet well documented. Notably, their ecological traits remain poorly understood, including the crucial root and soil interactions of *F. hodginsii*, which limit its planting area for ecological applications. Therefore, it is essential to comprehend the mechanisms underlying the ecological phenotypic differentiation among different *Fokienia hodginsii* provenances. Such knowledge can be useful in improving forest nursery management strategies for this species [19].

The phenotype of a woody plant is the outcome of its genetic background and intensive interaction with the local environment. Besides weather conditions, the soil ecosystems (i.e., bulk soil, rhizosphere, and endosphere) are critical to the growth of plants [20]. In particular, the rhizosphere and endosphere are where the relationships between soil nutrients, plant roots, and nutrient transport are focused, which are driven by both associated and endophytic microbes [21,22]. Numerous rhizosphere and endophytic microorganisms of *F. hodginsii* were identified that participate in the biogeochemical transformation of soil nutrients, including carbon, nitrogen, and phosphorus and their cycling, which are essential for soil improvement and plant phenomics [23–26]. Thus, rhizosphere and endosphere microorganisms are likely important causes for the differentiation of growth characteristics among different varieties within the same plant species.

To explore the microbial basis for ecological phenotypic differentiation of different *F. hodginsii* varieties, we examined the composition and variation of soil ecosystems in a homogeneous garden of the 11 main *F. hodginsii* provenances. High-throughput sequencing was utilized to detect and identify the rhizosphere and associated microbiota of *F. hodginsii* roots, while the nutrients of rhizosphere soil and roots were quantified to further study the relations among them. This work provides a fundamental advance in understanding how *F. hodginsii* associates with microbes to conduct its key ecological function (improving soil health and plant nutrient status). It also provides a reference database for selecting suitable provenances for differing soil conditions and plantation purposes and

for fostering the development and utilization of growth-promotion-related functional microorganisms [27,28].

2. Materials and Methods

2.1. Study Area and Sampling

The experimental site was located at the Nanjing State-owned forest farm in Zhangzhou, Fujian, China. The study area has a subtropical monsoon humid climate and lies in typical red soil territory. The mean annual temperature (MAT) is 21.2 °C. The annual sunshine hours (ASHs) are 1790 h. The annual precipitation (AP) is 1.7983 m. The annual average frost-free period is over 330 d. And the annual average relative humidity is 80%. The evergreen tree *Fokienia hodginsii* exhibits a distribution range spanning approximately from 22° to 28° N and 102° to 120° E. Characterized as a heliophilous species, *Fokienia hodginsii* possesses the ability to tolerate infertile soil and survive in environments with suboptimal growing conditions. Additionally, it demonstrates rapid growth rates in plantations. The seeds of *Fokienia hodginsii* were collected from the mother trees (23-year-old mature individuals) in 11 regions of China in 1996 (Table S1), and then placed in Dongxing Nursery of Yongtai County, Fuzhou City, for seedling raising.

In 1997, one-year-old seedlings (collected and propagated by the Fujian Academy of Forestry Science) with similar growth vigor were planted at the experimental site. A randomized complete block method was used in the experiment to set up the *F. hodginsii* plantation. There were a total of 5 blocks. The 11 provenances were randomly distributed within each block. Each provenance had 8 replicates and was planted in rows with 2 columns. *F. hodginsii* was planted by digging open holes and returning the topsoil without applying base fertilizer. All management practices and geological traits were similar among the blocks (Figure S1). Different varieties of *Fokienia hodginsii* material were collected in May 2021. First, the mean diameter at breast height (DBH) and mean tree height of all individuals were measured. Then, three healthy trees (n = 15 = 3 trees × 5 blocks) with similar average DBH and tree height were selected from various sources in each plot as sampling objects. The small hoe was used at the base of each plant for careful excavation. After the main roots of the trees were exposed, the exposed main roots were carefully excavated in the topsoil layer with a 0–0.2 m depth. The fine roots of *Fokienia hodginsii* with a diameter of less than 2 mm were cut in vitro [29], and the attachments on the fine roots were simply cleaned and loaded into two sterile bags. One bag was used for the determination of nutrient elements in the later stage. The other was immediately placed in an incubator with an ice bag for temporary storage. After being brought back to the laboratory, the soil and other impurities on the root were rinsed with 1 × phosphate-buffered saline (PBS), put into a centrifuge tube, and cryopreserved in an −80 °C ultra-low-temperature refrigerator for subsequent root microbial community sequencing analysis.

The rhizosphere soil of the same three strains of *Fokienia hodginsii* was collected. After removing the deciduous layer of *Fokienia hodginsii*, the upper covering soil was excavated layer by layer. After cutting off the fine root branches, the loose soil combined with the roots was shaken off. The soil still adhered to the fine roots was the rhizosphere soil. Part of it was collected with a small brush into a 50 mL centrifuge tube and immediately stored in a heat preservation box with an ice bag. After the sampling, it was immediately placed in a refrigerator at −80 ° C for sequencing analysis of the rhizosphere microbial community. Part of them were directly packed into sterile bags and brought back to the laboratory for the determination and analysis of nutrient elements. The rhizosphere soil obtained from each provenance of each block was mixed into 1 soil sample, and 5 blocks were repeated 5 times. Fine root [29] and rhizosphere soil [30] (<2 × 10^{-3} m in diameter) samples were collected from each plant by provenance, resulting in a total of 165 root or soil samples collected from the field. The 15 individuals per provenance were mixed together as one sample and randomly divided into three [31]. All samples were collected in sterilized

packages and immediately taken to the laboratory on dry ice for the quantification of nutrients, microbiome sequencing, and function prediction.

2.2. Quantification of Nutrients

After air-drying the rhizosphere soil samples, the plant roots, residues, and other impurities were removed. Then, they were mixed thoroughly and sieved through a 1.49×10^{-4} m sieve. The sieved soil samples were then packed into sealing bags for subsequent analysis. After washing with distilled water, the root samples were placed into separate envelopes and dried at 105 °C for 1 h and then at 80 °C for 24 h. The resulting powder was then crushed and screened through a 1.49×10^{-4} m sieve.

The total C and N contents of the soil and root samples were measured using a carbon–nitrogen analyzer (VARIO MAX, Elementar, Frankfurt, HE, Germany). After digestion of the soil and root samples, the total P, K, Ca, Mg, Fe, and Mn contents were determined using inductively coupled plasma–optical emission spectrometry (PE OPTIMA 8000, PerkinElmer, Waltham, MA, USA).

The soil digestion method was as follows: 1×10^{-4} kg of soil sample was weighed into a Teflon crucible, moistened with water, and then 10 mL of hydrochloric acid was added. The sample was heated at a low temperature (120 °C) on a hot plate to initially decompose the sample. When the volume was reduced to approximately 3 mL, the crucible was removed and allowed to cool slightly. Then, 5 mL of nitric acid, 5 mL of hydrofluoric acid, and 2 mL of perchloric acid were added, and the crucible was heated at a medium temperature (180 °C) for 1 h on a hot plate. The temperature was then increased to 220 °C to remove silica until the black residue was completely digested. When the contents of the crucible became viscous, it was removed and allowed to cool slightly. Then, 1% nitric acid solution was added to transfer the sample to a 50 mL volumetric flask, and the final volume was adjusted. The supernatant was then taken for testing.

The digestion method for root samples was as follows: 2×10^{-4} kg of plant sample was weighed into a digestion tube, and 5 mL of nitric acid and 1 mL of hydrogen peroxide were added. The digestion tube was placed on a digestion rack inside a digestion instrument (ETHOS UP, Milestone, Modena, Italy). After the digestion program was completed, the tube was placed in a fume hood, the cap was loosened to release gas, and the tube was carefully opened. The tube was rinsed multiple times with pure water, and filtered through quantitative filter paper, and the final volume was adjusted to 50 mL for testing.

2.3. Microbial Amplicon Sequencing

2.3.1. DNA Extraction and Quality Checking

Total DNA from each rhizosphere soil sample (11 provenances) was extracted using the E.Z.N.A. Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA), by following the manual's protocol. The concentration and quality of the genomic DNA were checked using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). All DNA samples were stored at −20 °C for subsequent experiments.

2.3.2. PCR Amplification

The bacterial 16S rRNA (V5–V7) gene regions were amplified in a nested PCR, by first using a primer combination of 799F (AACMGGATTAGATACCCKG) and 1391R (GACGGGCGGTGWGTRCA), followed by a second primer combination of 967F (CAACGCGAAGAACCTTACC) and 1391R (GACGGGCGGTGWGTRCA) [32–35]. For each soil sample, an 8-digit barcode sequence was added to the 5′ end of the forward/reverse primers (provided by Allwegene Company, Beijing, China). The first PCR was carried out on a Mastercycler Gradient (Eppendorf, Hamburg, HH, Germany) using a 2.5×10^{-5} L reaction volume that contained 1.25×10^{-5} L 2× Taq PCR MasterMix (Vazyme Biotech Co., Ltd, Nanjing, China), 3×10^{-6} L BSA (2×10^{-3} g·L^{-1}), 1×10^{-6} L forward primer (5×10^{-6} M), 1×10^{-6} L reverse primer (5×10^{-6} M), 2×10^{-6} L template DNA, and 5.5×10^{-6} L ddH2O,

under the following cycling conditions: 94 °C for 300 s, followed by 25 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s, with a final extension at 72 °C for 420 s. The second PCR conditions were the same as those of the first PCR. The PCR products were purified using an Agencourt AMPure XP Kit (Beckman Coulter, Inc., Brea, CA, USA).

The fungi ITS2 gene regions were also amplified in a nested PCR. The first primer combination was ITS1_KYO2-F (TAGAGGAAGTAAAAGTCGTAA) and ITS4_KYO3-R (TCCTCCGCTTATTGATATGC) and a second primer combination was ITS3_KYO2-F (GATGAAGAACGYAGYRAA) and ITS4_KYO3-R (TCCTCCGCTTATTGATATGC) [36,37]. The first PCR was carried out on a Mastercycler Gradient (Eppendorf, Hamburg, HH, Germany) using a 2.5×10^{-5} L reaction volume, same as above, with the following cycling conditions: 94 °C for 300 s, followed by 30 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s, with a final extension at 72 °C for 420 s. The second PCR conditions were the same as the cycling conditions. The ensuing PCR products were purified using an Agencourt AMPure XP Kit.

2.3.3. High-Throughput Sequencing

Deep sequencing was performed on an Illumina Miseq/Novaseq platform (Illumina, Inc., San Diego, CA, USA) by the Beijing Allwegene Technology Co., Ltd (Beijing, China). After running the samples, image analysis, base calling, and error estimation were conducted using the Illumina Analysis Pipeline v2.6 (Illumina, Inc., San Diego, CA, USA). The raw data were divided into different samples according to the barcode sequence through QIIME (v1.8.0) software [38]. Pear (v0.9.6) software was used to filter and splice raw data. The sequences were removed from consideration if they were shorter than 120 bp, had a low quality score (≤20), or contained ambiguous bases. During splicing, the minimum overlap setting was 10bp, and the mismatch rate was 0.1 [39]. After splicing, Vsearch (v2.7.1) software was used to remove sequences with lengths less than 230 bp and remove the chimeric sequence by the uchime method according to the Gold Database [40,41]. Sequence data associated with this project have been deposited in the NCBI Short Read Archive database (accession numbers: SRP414433, SRP415726).

2.4. Statistical Analysis

To comprehensively assess the diversity of microbial communities in the samples, we employed a range of alpha diversity and richness indices, including the Chao1, observed species, PD_whole_tree, Shannon, Simpson, and Invisimpson indices. These indices were used to determine the diversity and richness of the root bacteria in the roots of 11 *F. hodginsii* provenances. Likewise, fungi were determined using Mothur software (v.1.30.1). Principal coordinate analysis (PCoA) was carried out to measure how similar or dissimilar the samples were. In the ordination, each point corresponds to a sample, and the closer the distance between points, the more similar those samples are. Python (v2.7) software was used for the LEfSe analysis [42]. To screen the biomarkers with significant differences between the provenances, the rank sum test was used to detect the different species, and linear discriminant analysis (LDA) was applied for dimension reduction to obtain the LDA score. LEfSe was used to analyze the diversity among species, including the LDA value distribution histogram and evolutionary branching diagram (phylogenetic distribution). QIIME (v1.8.0) was used to generate the rarefaction curves and to calculate the richness and diversity indices based on the OTU information, whose plotting was undertaken in R (v3.6.0) software. Based on the results of taxonomic annotation and relative abundance, R was also used for bar-plot diagram analysis.

One-way analysis of variance (ANOVA) and analysis of variance of means ($p < 0.05$) were used to evaluate fine root nutrient content in SPSS 20.0 (SPSS Inc. Chicago, IL, USA). Correlation analysis (Spearman's) was carried out to identify the microbial taxa significantly associated with fine root nutrients. Functional predictions were made for C-, K-, and Ca-related microbial communities, resulting in different pathway results for the 11 provenances of *F. hodginsii*, among which the top five pathways with the most significant

differences were selected ($p < 0.05$). R statistical software, GraphPad Prism 8.0, and canoco5 were used to implement or visualize the correlations, cluster analysis, fine root nutrient content, principal component analysis (PCA), analysis of KEGG differences, the predicted KEGG functions, and redundancy analysis (RDA).

3. Results

3.1. Differences in Root Rhizosphere Soil (RS) Nutrient Contents Associated with Different Provenances

The C, N, P, K, Ca, Mg, Fe, and Mn contents of RS from different provenances ranged in concentration as follows: 1.23×10^{-2}–1.38×10^{-2} g·g^{-1}, 1.14×10^{-3}–1.51×10^{-3} g·g^{-1}, 1.70×10^{-4}–2.60×10^{-4} g·g^{-1}, 8.62×10^{-3}–1.314×10^{-2} g·g^{-1}, 1.00×10^{-3}–1.89×10^{-3} g·g^{-1}, 2.57×10^{-3}–3.42×10^{-3} g·g^{-1}, 3.00×10^{-3}–4.93×10^{-3} g·g^{-1}, and 7.00×10^{-5}–1.20×10^{-4} g·g^{-1}, respectively (Table S2). The variation in nutrient contents among the different RSs ranged from 3.17% to 24.64%, being most pronounced for Ca (highest percentage change) and least for N (lowest percentage). The content of Ca and Fe from different RSs differed significantly among provenances. Significantly higher contents of Ca ($p < 0.05$) could be detected in RS from HNDX, JXSY, and GDSX compared with other provenances, and likewise, a higher Fe content in the RS from FJCT, FJGT, and FJXY was noted.

3.2. Diversity and Construction of Root Rhizosphere Microbes (RMs) Associated with Different Provenances

Overall, 6,557,201 clean reads were sequenced from the RM samples of 11 provenances, which were then assigned to 16 568 OTUs (operational taxonomic units) of bacteria and 5854 OTUs of fungi (Table S3). All these bacteria belonged to 36 phyla, 83 classes, 201 orders, 300 families, 508 genera, and 756 species, while the fungi belonged to 18 phyla, 53 classes, 158 orders, 349 families, 801 genera, and 1440 species. The dominant bacterial phyla were Acidobacteriota, Actinobacteriota, Proteobacteria, and Chloroflexi, which together accounted for 80% of the total species number (Figure S2A). For fungi, the dominant phyla were Ascomycota, Unidentified, and Basidiomycota (Figure S2B). No obvious differences could be detected from intragroup and intergroup analyses of bacterial communities and likewise at the intragroup level of fungi, but a significant intergroup difference was found for fungal communities (mainly due to the richness of Basidiomycota).

3.3. Connections between Root Rhizosphere Soil Nutrients and Microbes in Regard to Different Provenances

No obvious correlations could be observed between the diversity of RMs and the nutrient content of RSs associated with the different provenances. At the phylum level, redundancy analysis of RMs and RS nutrients showed the following (Figure 1A,B): The cumulative variance of root nutrients for bacteria and fungi was 54.95% and 65.44%, and the P content explained most of that in both bacteria and fungi, with a significant correlation ($p < 0.05$). For bacteria, the RS nutrients (P, C, K, Ca, Mg, Fe) were positively correlated with Crenarchaeota, Verrucomicrobiota, Desulfobacterota, and NB1-j yet negatively correlated with Abditibacteriota and Dependentiae ($p < 0.05$). Meanwhile, the RS nutrients (P, K, Ca, Mg, and Fe) were positively correlated with the richness of fungal communities (Zoopagomycota, Ascomycata, Glomeromycota, and Kickxellomycota) and negatively correlated with GS01 and Basidiobolomycota ($p < 0.05$).

Moreover, the correlation between the nutrient contents of RS and environmental factors of the origin provinces of different provenances was also examined (Figure 1C). This revealed that longitude was negatively correlated with the P, K, Mg, and Fe content of RS, while annual sunshine hours were negatively correlated with K, Mg, Fe, and Ca, whereas elevation was positively correlated with K and Ca ($p < 0.05$).

The correlation between the richness of different RMs and environmental factors of the original provinces of different provenances was also explored. The proportion of total

variance explained in the correlation between environmental factors and richness of bacterial (Figure 1D) and fungal (Figure 1E) communities was 99.14% and 93.21%, respectively. Both bacterial phyla SAR324 clade Marine Group B and Cyanobacteria and the fungal phylum Basidiomycota were positively correlated with longitude and annual sunshine hours but negatively correlated with the elevation of different provenances.

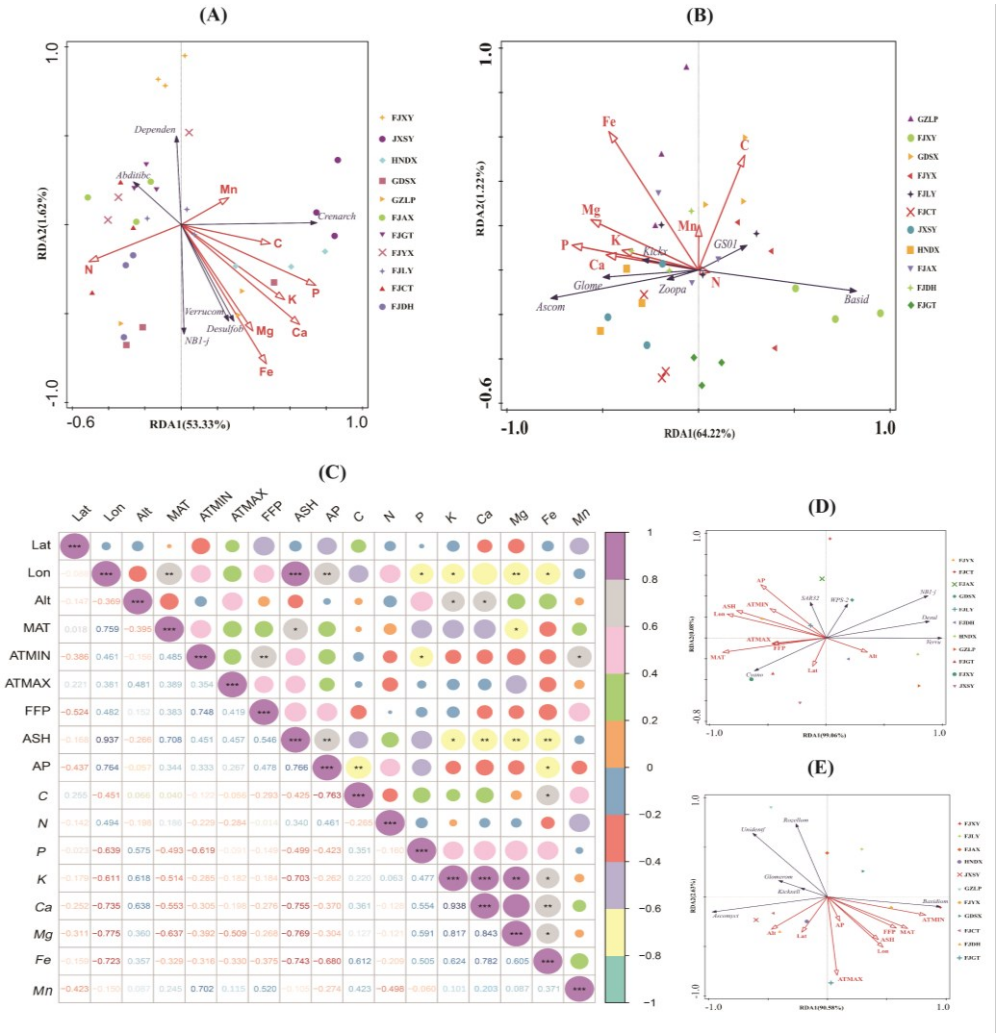

Figure 1. Correlation between rhizosphere soil nutrients and microorganisms regarding the different provenances of *Fokienia hodginsii*. Shown are correlations between rhizosphere soil nutrients and the assembled bacterial (**A**) or fungi (**B**) community, visualized in an RDA plot. The correlations between environmental factors of the original provinces and rhizosphere soil nutrients were plotted according to their Pearson's r correlation coefficient (**C**); for each circle, its number and area represent the r value; the *, **, and *** denote the probability that the correlation between environmental factors of the original provinces and rhizosphere soil nutrients in the sample data occurred by chance at $0.01 < p < 0.05$, $0.001 < p < 0.01$, and $p < 0.001$, respectively. Meanwhile, correlations between environmental factors of original provinces and rhizosphere soil bacteria (**D**) or fungi (**E**) were plotted in an RDA. Lat, Lon, Alt, MAT, ATMIN, ATMAX, AT, FFP, ASH, and AP are, respectively, the mean latitude, longitude, elevation, annual temperature, annual minimum temperature, annual maximum temperature, ≥10 °C-accumulated temperature, frost-free period, annual sunshine hours, and annual precipitation.

3.4. Diversity and Construction of Associated Microbiota (AM) in Different Provenances of F. hodginsii

For each *F. hodginsii* provenance, its AM was measured via high-throughput sequencing. The alpha diversity indices (chao1, observed_species, PD_whole_tree, and Shannon) were used to compare differences in fungal and bacterial community diversity among

samples (Figure 2A). Among the 11 provenances of *F. hodginsii*, the three provenances, FJAX, FJYX, and FJXY, exhibit the highest overall diversity in the bacterial community. FJAX has the highest diversity in terms of the chao1 index, with species observed and PD_whole_tree. The highest diversity for the two indexes came from FJYX, while FJXY had the highest diversity in the Shannon index (Figure 2A). For fungal diversity, GZLP and FJGT were the two provenances with the highest overall diversity. GZLP had the highest diversity in terms of chao1 and observed_species, while FJGT had the highest diversity in terms of PD_whole_tree and Shannon index (Figure 2B). The associated bacterial communities of 11 trophic types of *F. hodginsii* are composed of 37 phyla and 543 genera, with Proteobacteria being the dominant phylum across all samples. The relative abundance of Proteobacteria in 11 different provenances ranges from 4.42% to 51.76% (Figure 2C). The endophytic fungi found in different root samples belong to 12 phyla and 597 genera, with P_Ascomycota being the dominant fungal phylum in all 11 provenances of *F. hodginsii*, exhibiting a relative abundance ranging from 44.31% to 85.77%. The relative community abundance of the first six fungal genera is shown in Figure 2D, where P_ Basidiomycota occupies a secondary position in GZLP, JXSY, GDSX, HNDX, FJCT, FJLY, FJAX, FJDH, FJGT, FJXY, and FJYX root samples, with a relative abundance ranging from 9.59% to 48.01%.

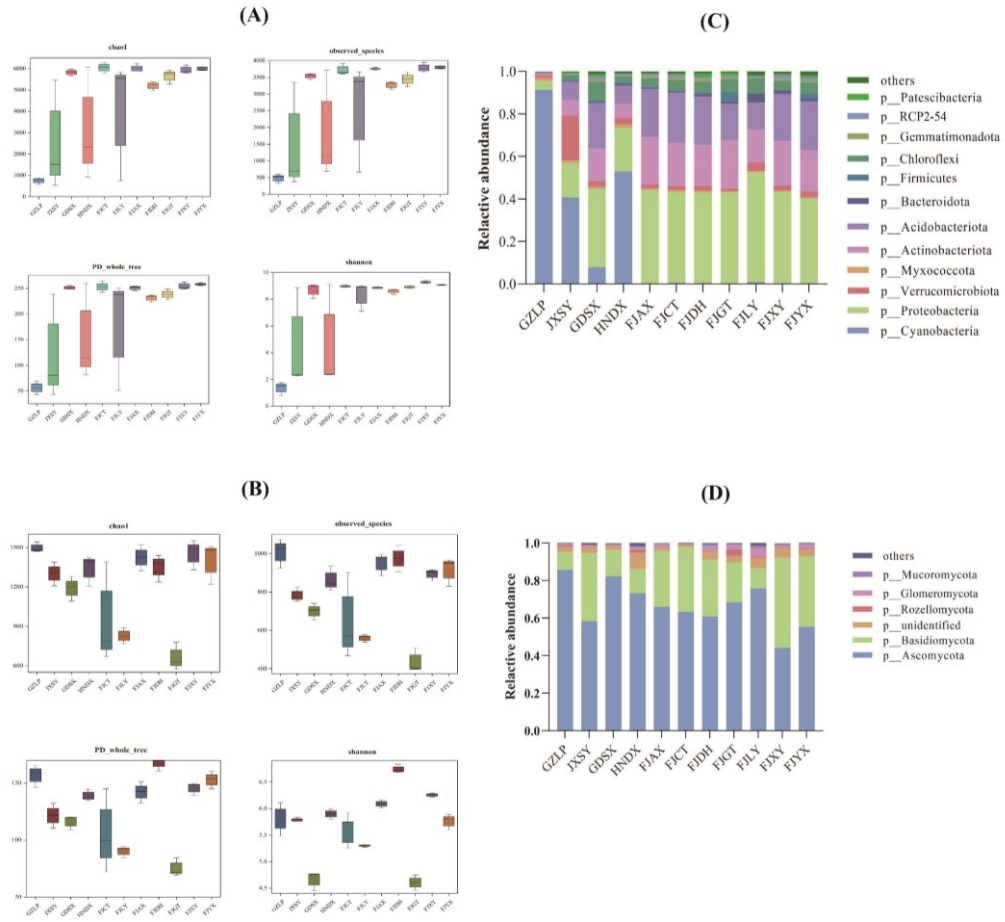

Figure 2. Diversity and assembly of associated microbiota in different provenances of *Fokienia hodginsii*. Alpha diversity of associated bacterial (**A**) and fungal (**B**) communities in different provenances was compared and shown using four indices: chao1, observed_species, PD_whole_tree, and Shannon index. Assembly of bacterial (**C**) and fungal (**D**) communities of different samples presented at the taxonomic levels of phylum.

3.5. Links between Root-Associated Microbiota and Nutrient Element Contents in Different Provenances of F. hodginsii

To uncover linkages between element concentration and the associated microorganisms of roots of *F. hodginsii* types, Spearman correlations were performed. Regarding associated bacterial communities (Figure 3A), (1) the C, K, and Mg contents were all significantly correlated with unidentified microorganisms ($p < 0.05$, $\rho > 0.8$); (2) both p and Fe contents were significantly correlated with Actinospica ($p < 0.05$, $\rho > 0.8$); and (3) the N, Ca, and Mn contents were significantly correlated with Acidicaldus, Acidothermus, and Brevundimonas, respectively ($p < 0.05$, $\rho > 0.8$). Concerning associated fungal communities (Figure 3B), the C, N, P, K, Ca, Mg, Fe, and Mn contents were significantly correlated with Hymenochaete, Talaromyces, Pestalotiopsis, Vermispora, unidentified, Neonothopanus, Elaphomyces, and Pezicula, respectively ($p < 0.05$, $\rho > 0.8$).

Significant differences could be detected between the abundances of nutrient-related associated bacterial communities from different provenances of *F. hodginsii* (Figure 3C). The abundance of N- and Fe-related associated bacteria in FJCT was significantly higher than that in other provenances. The abundance of C- and N-related associated bacteria in GZLP and JXSY was significantly higher than the others, respectively. For associated fungi (Figure 3D), however, the C-, P-, K-, and Mg-related associated fungal communities, respectively, had higher abundances in FJCT, FJLY, FJGT, and FJXY than the others. To delve further into the role of root-associated microbiota in different types of *F. hodginsii*, we compared the metabolic pathways of 11 provenances and found significant differences among them (using the Kruskal–Wallis test, $p < 0.05$), with the most notable differences observed in the C-, K-, and Ca-related microbial communities. Through functional prediction, we identified hundreds of pathways and screened 15 different pathways related to plant–microbe interactions (five pathways related to C, K, and Ca elements each) in both bacterial and fungal communities. We then analyzed the relative abundance of microbial communities in different provenances under these pathways (Figures 3E and S3). Our analysis revealed that, in the bacterial community, the synthesis and degradation of ketone bodies pathway showed the most significant difference among the galactose metabolism, ascorbate and aldarate metabolism, fatty acid biosynthesis, and phenylalanine metabolism pathways. Furthermore, it was significantly higher in GDSX, FJCT, FJLY, FJAX, FJDH, FJGT, FJXY, and FJYX than in GZLP, JXSY, and HNDX. In the C-related fungal community, our pathway analysis revealed that Fasyn-elong-pwy was the most significantly different, while Leu-deg2-pwy was the most significant in the Ca-related fungal community.

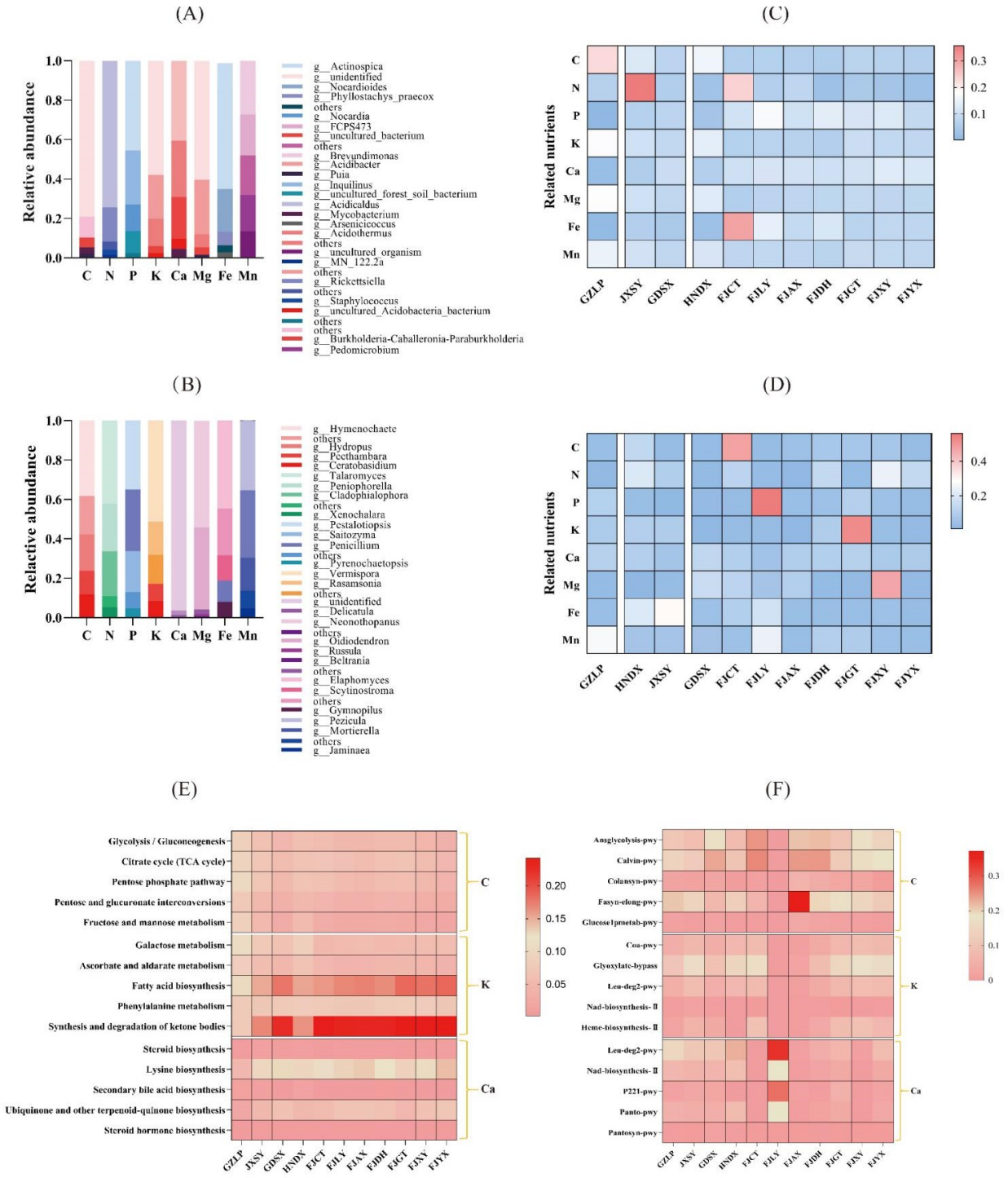

Figure 3. Critical associated microbiota drives nutrient uptake in different provenances of *Fokienia hodginsii*. Spearman correlations were tested between root nutrient elements and the abundance of bacterial or fungi community taxa. Relative abundances of critical associated bacterial (**A**) and fungal (**B**) communities significantly highly correlated with the content of different nutrient elements are presented at the taxonomic level of the genus ($p < 0.05$, $\rho > 0.8$). The relative abundances of different nutrient elements correlated with bacterial (**C**), and fungal (**D**) communities are shown at the taxonomic level of genus. To elucidate the gene function of the associated microbial communities exclusive to distinct provenances of *F. hodginsii*, we conducted functional prediction analysis on the bacterial (**E**) and fungal (**F**) communities in the root systems of *F. hodginsii* and found significant differences among the C-, K-, and Ca-related microbial communities.

4. Discussion

Common garden experiments can control for the influence of climatic factors and site conditions on different provenances, thereby revealing differences in nutrient utilization strategies of *F. hodginsii* trees due to their interspecific genetic background, which is fundamental to predicting the phenotypes of differing provenances in heterogeneous environments [43,44]. In the current study, we first demonstrated that the greatest difference in soil among the 11 provenances of *F. hodginsii* is the amount of Ca and Fe. Through the analysis of rhizosphere soil nutrients and microbiota of different provenances of *F. hodginsii*, we find that microbial community diversity is not significantly correlated with soil nutrient features, although some specific functional microbial communities can drive soil nutrient differentiation.

Based on the root nutrients, the 11 provenances of *F. hodginsii* can be grouped into three different nutrient types [45] (Figure S4; Table S4). Stark differences could be detected in the C, K, and Ca contents among these nutrient types of *F. hodginsii*. Provenances within Clade II have much lower C, K, and Ca uptake capacity than either Clade I or Clade III. In a PCA, a dataset is considered representative when the cumulative proportion of variance of the principal components exceeds 80%. In this study, the eigenvalues of the top two principal components (C and N) were all greater than 87%. Their cumulative proportion of explained variance reached 100%, meaning that the top two independent principal (C and N) components captured 100% of the variation in the eight quality-related traits. Similar to the rhizosphere soil, the diversity of root-associated microbial communities was not related to changes in nutrient element concentrations of *F. hodginsii*; instead, some microbial communities significantly correlated with N and P contents of the root systems formed distinct nutrient concentration change patterns. Therefore, this study identified differences in soil improvement and nutrient absorption among different provenances of *F. hodginsii* and successfully screened and described key microbial communities that influence its soil improvement and nutrient element changes.

The growth traits of trees are shaped by both environmental and genetic factors [46,47]. This study focused on 11 provenances of *F. hodginsii*, including the local provenances within Fujian Province and the non-local provenances from other provinces. All individuals were 23-year-old mature individuals with root growth essentially stagnant; hence, the interaction between the soil and their root system also tended to be stable in this garden environment [48–50]. Interestingly, the exotic *F. hodginsii* provenances in this study (i.e., GZLP, HNDX, JXSY, and GDSX) have higher soil nutrient content (C, P, K, Ca, Mg, Fe, and Mn) than native provenances or the already widely used soil modification species in Fujian (like *P. massoniana* and *Casuarina equisetifolia*), which suggest they could be more suitable for local cultivation [51,52]. We also found that the rhizosphere soil nutrient characteristics of *F. hodginsii* were negatively correlated with the annual sunshine duration (ASH) of the original provenance, but their DBH was highly positively correlated with ASH (Figure 1C). It is well known that sufficient sunlight is crucial to the growth of *F. hodginsii* such that in areas with relatively less sunlight, its growth is inhibited, which could require greater dependence on plant traits for improving local soil nutrient conditions under the same circumstances to augment its living space [53,54]. This leads to the idea that the *F. hodginsii* provenances originating from places north of Fujian harbor have a more powerful ability to enrich most soil nutrient elements in Fujian.

Clade I can more efficiently utilize N, while Clade III is more efficient in the absorption and conversion of P (Figure S4). However, in terms of soil nutrient characteristics, the N content of the rhizosphere soil of Clade I did not surpass that of the other two clades, and the P content of Clade III's rhizosphere soil did not differ significantly from the other clades. The study clustered the nutrients in the rhizosphere soil (Figure S3A) into two different categories, whereby Clade I and Clade III were clustered into the same single category. This result indicates the soil conditions of Clade I and Clade III are similar; thus, the difference in nutrient utilization comes from the plant itself, while the nutrient contents of Clade II are determined by soil circumstances. In their long process of evolution,

the exotic provenances of *F. hodginsii* (Clade I and Clade III) have developed their specific root phenotype, associated microbiota, and nutrient characteristics adapted to the soil environment of their own original provenance [55]. Normally, the duration or intensity of environmental changes does not reach the threshold for causing the adaptation of exotic provenances [56]. As such, these functional traits will likely not benefit the plant in exotic soil environments [57]. Rhizosphere microorganisms are critical to the above phenomena, being key groups that first perceive environmental changes and participate in soil nutrient cycling [58,59]. Here, the rhizosphere microorganisms of exotic provenances were sensitive to the environmental difference. Yet due to the lack of key functional microbiota (at least the Chloroflexi) in the roots of those exotic provenances, they failed to benefit from the nutrient-enriched soil environment. In future work, it will be pivotal to identify the missing critical functional microbiota that can augment the growth of exotic provenances by promoting the interaction between *F. hodginsii* and the soil environment.

The diversity of microorganisms is a key consideration for how plants benefit from their soil environment [60]. However, in this study, the diversity of either the rhizosphere or associated microbial communities is not related to nutrient contents, which means that in addition to nutritional characteristics, there may be other phenotypic differences among the 11 provenances, such as in their plant stress resistance traits [61–63], plant biomass [64,65], and root structure. Nevertheless, we did find that some associated bacteria are highly correlated with soil and plant nutrient characteristics. Among them, the abundance of Caulobacter [66], Peniophora [67,68], Nocardia [69], Lysinibacillus [70], and Brevundimonas [71] were significantly correlated with C, K, Ca, Mg, Fe, and Mn contents of plant roots, all of which are important microorganisms involved in respiratory metabolism and energy decomposition processes. Based on the differences in the abundance of element-related microbial communities among different provenances, N-related bacteria communities were significantly higher in JXSY than in other bacterial communities and provenances, indicating that *F. hodginsii* from JXSY may prioritize meeting its own N element requirements through root-associated bacteria. Other element-related bacterial communities were less abundant with smaller changes in element concentrations, suggesting weaker adaptation of this provenance to low-P conditions in Fujian. In addition, K-related fungal communities were significantly higher in FJGT than in other provenances, and K is involved in physiological and biochemical processes such as the activation of plant enzymes, photosynthesis, and sugar metabolism, which may enhance the utilization of other elements by plant roots and promote the growth of *F. hodginsii*. Moreover, compared with other provenances, FJLY had a higher relative abundance of fungal communities related to P, which corresponds to the low-P soil conditions in Fujian and may contribute to the stronger adaptability and higher survival rate of this provenance in the local area.

According to Figure S5, the abundance of Crenarchaeota [72,73], Verrucomicrobiota [74–76], and Desulfobacterota [77], all of which are functional microbes in the decomposition of organic matter and minerals, is significantly positively correlated with the soil contents of C, P, K, Ca, Mg, Fe, and Mn. The phylum Abditibacteriota, whose members are nitrate nitrogen and ammoniacal nitrogen enrichment-related bacteria, was positively correlated in abundance with soil N content enrichment. Meanwhile, among the three *F. hodginsii* clades, the abundance of Abditibacteriota was higher in Clade I, suggesting the latter's N utilization efficiency was higher [78]. By contrast, the enriched abundance of Proteobacteria [79], whose members are P transport-related bacteria, suggests that Clade III has a higher P uptake efficiency. Of interest is the relatively high abundance of Chloroflexi in the native provenances of *F. hodginsii* (Clade II); this type of bacteria can be significantly induced and enriched by low P conditions [80,81], being consistent with the low-P soil conditions in Fujian Province. This hints that Chloroflexi might be instrumental to *F. hodginsii*'s sensitivity to soil environments low in P [82–84].

In this study, functional predictions were made for microbial communities significantly correlated with C, K, and Ca. Based on the bacterial prediction results, Bacillus ce-

reus induced a large number of genes related to glycolysis/gluconeogenesis, starch metabolism, and oxidative stress [85], which may affect carbon absorption by influencing the main hormones involved in plant defense. Differences in bacterial species between Eurotiomyetes and Sordariomycetes were mainly reflected in differences in the amino acid metabolism and ABC transporter pathways [86], which may result in differences in K element content. Proteobacteria activated pathways such as ABC transporters, energy metabolism, and fatty acid metabolism [87], affecting calcium absorption. Meanwhile, based on functional predictions of fungi, Chloroflexi may indirectly affect carbon content in plants by regulating the glycolysis/gluconeogenesis metabolic pathway to influence soil carbon component water changes [88].

According to Figure S5, although dozens of metabolic pathways of associated microbes were predicted to have significant differences among provenances, only five of them were confirmed by genetic expression patterns from metagenomics. Among the above five metabolic pathways, the phenylalanine metabolism pathway was highly activated in both Clade I and Clade III and may be involved in N and P uptake through promoting flavonoid synthesis and photosynthesis [89]. Meanwhile, the histidine metabolism pathway was highly activated in Clade II compared to the others, which may promote the Ca uptake through the regulation of histidine-rich calcium-binding protein (HRC) [90]. Notably, the other three metabolic pathways are closely linked to the interactions between host plants and associated microbes. The biosynthesis of siderophore-group nonribosomal peptides plays a significant role in hindering the growth of plant pathogenic microorganisms through the secretion of siderophores [91]. Selenocompound metabolism assists plants in synthesizing proteins and enzymes essential for photosynthesis and contributes to antioxidant responses [92]. Glycerophospholipid metabolism is responsible for maintaining the integrity and stability of cell membranes, aiding plants in resisting external stress and pathogen invasion [93]. In general, both the functional microbes and genes of the above five pathways in Clade II were highly enriched compared to the other two clades, which indicated that native provenances of *F. hodginsii* have much more intense interaction with root-associated microbes.

5. Conclusions

For the native provenances of *F. hodginsii*, their economic traits (i.e., DBH and tree height) (Table S5) are better than the other exotic provenances. Also, native provenances are more sensitive to local soil conditions, so they should benefit more from human interventions (such as fertilization), rendering them more suitable for artificial cultivation. The exotic provenances (GZLP, HNDX, JXSY, and GDSX) are characterized by a higher soil nutrient content, and their growth is less affected by the soil environment, leaving them better suited for the ecological transformation of forest stands and soil improvement. Also, these exotic provenances share the characteristics of pioneer tree species that often establish and grow well in challenging woodland environments. From the perspective of improving soil conditions, exotic provenances of *F. hodginsii* trees are more effective than the main local pioneer *P. massoniana* in southern Fujian.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f15061005/s1.

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References

1. Cai, N.H.; Tang, J.R.; Li, Y.Q.; Chen, S.; Chen, L.; Xu, Y.L.; Li, G.Q. Effect of shading on growth and biomass of *Fokienia hodginsi* seedling. *J. Fujian Agric. For. Univ. (Nat. Sci. Ed.)* **2022**, *44*, 1305–1313.
2. Chen, Z.S. Study on Physical Properties and Mechanical of *Fokienia hodginsi*. *J. For. Environ.* **1999**, *3*, 32–35.
3. Lin, C.F.; Li, Z.; Niu, Z.P.; Zhang, Y.L.; Han, Y.G.; Chen, G.S. Litterfall nutrient dynamics in *Fokienia hodginsi* plantation. *J. Fujian Agric. For. Univ.* **2005**, *1*, 63–66.
4. You, J.P. Water holding characteristics and ecological stoichiometric characteristics of carbon, nitrogen and phosphorus in litter of *Fokienia hodginsii* mixed forest. *For. Sci. Technol.* **2022**, *47*, 24–27.
5. Yang, G.C. Study on the growth effect of different mixed ratio of *Fokienia hodginsii* and Pinus massoniana. *Green Technol.* **2022**, *24*, 99–102.
6. Li, B.J.; Chen, Q.; Wang, X.X.; Rong, J.D.; Chen, L.G.; Zheng, Y.S. Differences in growth and nutrients between pure and mixed forests of *Fokienia hodginsii* at different ages. *Northwest Bot. J.* **2022**, *42*, 694–704.
7. Tóth, Á. *Bursaphelenchus xylophilus*, the pinewood nematode: Its significance and a historical review. *Acta Biol. Szeged.* **2011**, *55*, 213–217.
8. Liu, Q.; Wei, Y.; Xu, L.; Hao, Y.; Chen, X.; Zhou, Z. Transcriptomic Profiling Reveals Differentially Expressed Genes Associated with Pine Wood Nematode Resistance in Masson Pine *(Pinus massoniana* Lamb.). *Sci. Rep.* **2017**, *7*, 4693.
9. Ponpandian, L.N.; Rim, S.O.; Shanmugam, G. Phylogenetic characterization of bacterial endophytes from four *Pinus* species and their nematicidal activity against the pine wood nematode. *Sci. Rep.* **2019**, *9*, 12457.
10. Liu, F.; Su, H.; Ding, T.; Huang, J.; Liu, T.; Ding, N.; Fang, G. Refined Assessment of Economic Loss from Pine Wilt Disease at the Subcompartment Scale. *Forests* **2023**, *14*, 139.
11. Yin, Q.; Chen, S.; Guo, W.; Huang, Y.; Huang, Y.; Zhou, R.; Fan, Q.; Liao, W. Pronounced genetic differentiation in *Fokienia hodginsii* revealed by simple sequence repeat markers. *Ecol. Evol.* **2018**, *8*, 10938–10951.
12. Zheng, W.; Gou, X.; Su, J.; Fan, H.; Yu, A.; Liu, W.; Deng, Y.; Manzanedo, R.D.; Fonti, P. Physiological and Growth Responses to Increasing Drought of an Endangered Tree Species in Southwest China. *Forests* **2019**, *10*, 514.
13. Chen, F.; Mu, J.; Huang, L.; Cui, X.Y.; Jin, Y.; Zhang, L. Features and succession analysis of *Fokienia hodginsii* communities in Sanchahe of Xishui National Nature Reserve, Guizhou Province. *South. For. Sci.* **2018**, *46*, 8–12.
14. Li, Y.; Li, X.; Zhao, M.H.; Pang, Z.Y.; Wei, J.T.; Tigabu, M.; Chiang, V.L.; Sederoff, H.; Sederoff, R.; Zhao, X.Y. An Overview of the Practices and Management Methods for Enhancing Seed Production in Conifer Plantations for Commercial Use. *Horticulturae* **2021**, *7*, 252.
15. Huang, S.J.; Rong, J.D.; Zhang, L.H.; Yang, Y.; Jiang, J.J.; Zheng, Y.S. Research summarization of *Fokienia hodginsii*. *Fujian For. Sci. Technol.* **2013**, *40*, 236–242.
16. Chen, Q.; Huang, X.; Jiang, D.H.; Ren, K.; Rong, J.D.; Chen, L.G.; Zheng, Y.S. Effects of shading on growth and biomass of *Fokienia hodginsii* seedlings. *J. Fujian Agric. For. Univ.* **2020**, *49*, 796–802.
17. Zheng, R.H.; Yang, Z.W.; Liang, H.Y.; Xiao, X.X.; Li, W.Y. A Study on the provenance test of *Fokienia hodginsi* in seedling stage. *J. Fujian For. Univ.* **2001**, *1*, 40–44.
18. Yang, Z.W.; Zheng, R.H.; Xiao, X.X.; Hou, B.X.; Cheng, Z.H.; Zeng, Z.G.; Xiao, F.M.; Li, X.M. A study on the genetic variation of seedling growth and root traits among provenance of *Fokienia hodginsii*. *J. Nanjing For. Univ.* **2001**, *3*, 26–30.
19. Kuppler, J.; Albert, C.; Ames, G.; Armbruster, W.; Bönisch, G.; Boucher, F. Global gradients in intraspecific variation in vegetative and floral traits are partially associated with climate and species richness. *Glob. Ecol. Biogeogr.* **2020**, *29*, 10.
20. Cheng, Z.; Lei, S.; Li, Y.; Huang, W.; Ma, R.; Xiong, J.; Zhang, T.; Jin, L.; Haq, H.U.; Xu, X.; et al. Revealing the Variation and Stability of Bacterial Communities in Tomato Rhizosphere Microbiota. *Microorganisms* **2020**, *8*, 170.
21. Liu, S.; He, F.; Kuzyakov, Y.; Xiao, H.; Hoang, D.T.T.; Pu, S.; Razavi, B.S. Nutrients in the rhizosphere: A meta-analysis of content, availability, and influencing factors. *Sci. Total Environ.* **2022**, *826*, 153908.
22. White, J.F.; Kingsley, K.L.; Zhang, Q.; Verma, R.; Obi, N.; Dvinskikh, S.; Elmore, M.T.; Verma, S.K.; Gond, S.K.; Kowalski, K.P. Review: Endophytic microbes and their potential applications in crop management. *Pest Manage. Sci.* **2019**, *75*, 2558–2565.
23. Fadiji, A.E.; Ayangbenro, A.S.; Babalola, O.O. Organic Farming Enhances the Diversity and Community Structure of Endophytic Archaea and Fungi in Maize Plant: A Shotgun Approach. *J. Soil Sci. Plant Nutr.* **2020**, *20*, 2587–2599.
24. Bulgarelli, D.; Garrido-Oter, R.; Münch, P.C.; Weiman, A.; Dröge, J.; Pan, Y.; McHardy, A.C.; Schulze-Lefert, P. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe* **2015**, *17*, 392–403.
25. Hacquard, S.; Garrido-Oter, R.; González, A.; Spaepen, S.; Ackermann, G.; Lebeis, S.; McHardy, A.C.; Dangl, J.L.; Knight, R.; Ley, R.; et al. Microbiota and Host Nutrition across Plant and Animal Kingdoms. *Cell Host Microbe* **2015**, *17*, 603–616.
26. Verma, P.; Yadav, A.; Kumar, V.; Singh, D.P.; Saxena, A.K. *Beneficial Plant-Microbes Interactions: Biodiversity of Microbes from Diverse Extreme Environments and Its Impact for Crop Improvement Plant-Microbe Interactions in Agro-Ecological Perspective*; Springer: Cham, Switzerland, 2017, pp. 543-580.

27. Liu, F.; Hewezi, T.; Lebeis, S.L.; Pantalone, V.; Grewal, P.S.; Staton, M.E. Soil indigenous microbiome and plant provenances cooperatively modify soybean rhizosphere microbiome assembly. *BMC Microbiol.* **2019**, *19*, 201.
28. Chang, J., Sun, Y., Tian, L., Ji, L., Luo, S., Nasir, F., Kuramae, E.E., Tian, C. The Structure of Rhizosphere Fungal Communities of Wild and Domesticated Rice: Changes in Diversity and Co-occurrence Patterns. *Front. Microbiol.* **2021**, *12*, 610–823.
29. Chen, F.S.; Niklas, K.J.; Liu, Y.; Fang, X.M.; Wan, S.Z.; Wang, H. Nitrogen and phosphorus additions alter nutrient dynamics but not resorption efficiencies of Chinese fir leaves and twigs differing in age. *Tree Physiol.* **2015**, *35*, 1106–1117.
30. Khan, A.; Wang, Z.; Chen, Z.; Bu, J.; Adnan, M.Y.; Zhang, M. Investigation of soil nutrients and associated rhizobacterial communities in different sugarcane provenances in relation to sugar content. *Chem. Biol. Technol. Agric.* **2021**, *8*, 59.
31. Jiang, O.Y.; Li, L.; Duan, G.L.; Gustave, W.; Zhai, W.W.; Zou, L.N.; An, X.; Tang, X.J.; Xu, J.M. Root exudates increased arsenic mobility and altered microbial community in paddy soils. *J. Environ. Sci.* **2023**, *127*, 410–420.
32. Walker, J.J.; Pace, N.R. Phylogenetic composition of rocky mountain endolithic microbial ecosystems. *Appl. Environ. Microbiol.* **2007**, *73*, 3497–3504.
33. Dos Santos, M.; Kavamura, V.; Reynaldo, E.; Souza, D.; Da Silva, E.; May, A. Bacterial structure of agricultural soils with high and low yields. *J. Plant Pathol. Microbiol.* **2017**, *8*, 405.
34. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583.
35. Beckers, B.; Op De Beeck, M.; Thijs, S.; Truyens, S.; Weyens, N.; Boerjan, W. Performance of 16s rDNA primer pairs in the study of rhizosphere and endosphere bacterial microbiomes in metabarcoding studies. *Front. Microbiol.* **2016**, *7*, 650.
36. Toju, H.; Tanabe, A.S.; Yamamoto, S.; Sato, H. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLoS ONE* **2012**, *7*, e40863.
37. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR—Protocols and Applications—A Laboratory Manual*; Academic Press: Cambridge, MA, USA, 1990; pp. 315–322.
38. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods.* **2010**, *7*, 335–336.
39. Zhang, J.; Kobert, K.; Flouri, T.; Stamatakis, A. PEAR: A fast and accurate Illumina Paired-End read merger. *Bioinformatics* **2014**, *30*, 614–620.
40. Rognes, T.; Flouri, T.; Nichols, B.; Quince, C.; Mahé, F. VSEARCH: A versatile open source tool for metagenomics. *PeerJ* **2016**, *4*, e2584.
41. Pruesse, E.; Quast, C.; Knittel, K.; Fuchs, B.M.; Ludwig, W.; Peplies, J.; Glöckner, F.O. SILVA: A comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* **2007**, *35*, 7188–7196.
42. Segata, N.; Izard, J.; Waldron, L.; Gevers, D.; Miropolsky, L.; Garrett, W.S.; Huttenhower, C. Metagenomic biomarker discovery and explanation. *Genome Biol.* **2011**, *12*, R60.
43. Ballentine, B.; Greenberg, R. Common garden experiment reveals genetic control of phenotypic divergence between swamp sparrow subspecies that lack divergence in neutral provenances. *PLoS ONE* **2010**, *5*, e10229.
44. Yuan, S.; Guo, C.; Ma, L.; Wang, R.Z. Environmental conditions and genetic differentiation: What drives the divergence of coexisting Leymus chinensis ecotypes in a large-scale longitudinal gradient. *J. Plant Ecol.* **2016**, *9*, 616–628.
45. Wendling, M.; Büchi, L.; Amossé, C.; Sinaj, S.; Walter, A.; Charles, R. Influence of root and leaf traits on the uptake of nutrients in cover crops. *Plant Soil* **2016**, *409*, 419–434.
46. Gloy, J.; Herzschuh, U.; Kruse, S. Evolutionary adaptation of trees and modelled future larch forest extent in Siberia. *Ecol. Modell.* **2023**, *478*, 110278.
47. Guevara, M.Á.; Sánchez-Gómez, D.; Vélez, M.D.; de María, N.; Díaz, L.M.; Ramírez-Valiente, J.A.; Mancha, J.A.; Aranda, I.; Cervera, M.T. Epigenetic and Genetic Variability in Contrasting Latitudinal *Fagus sylvatica L.* provenances. *Forests* **2022**, *13*, 1971.
48. Wang, H.; Lin, S.; Dai, J.; Ge, Q. Modeling the effect of adaptation to future climate change on spring phenological trend of European beech (*Fagus sylvatica* L.). *Sci. Total Environ.* **2022**, *846*, 157540.
49. Rabarijaona, A.; Ponton, S.; Bert, D.; Ducousso, A.; Richard, B.; Levillain, J.; Brendel, O. Provenance Differences in Water-Use Efficiency Among Sessile Oak Populations Grown in a Mesic Common Garden. *Front. For. Glob. Change* **2022**, *9*, 199.
50. Kawecki, T.J.; Ebert, D. Conceptual issues in local adaptation. *Ecol. Lett.* **2004**, *7*, 1225–1241.
51. Zhou, Z.C.; Xie, Y.R.; Jin, G.Q.; Wu, J.F.; Chen, Y. Genetic response of pinus massoniana provenances to phosphorus supply and nutrient characteristics of their rhizosphere soil. *Sci. Silvae Sin.* **2003**, *6*, 62–67.
52. Ye, G.F.; Zhang, Y.; Luo, M.J.; Xu, j.S.; Yu, X.J. Effects of low phosphorus stress on nutritional status of *Casuarina equisetifolia* provenances at seedling stage. *Straits Sci.* **2008**, *22*, 51–52+59.
53. Ling, Y.T. Analysis on the effect of release-thinning and pruning to mixed forest of *Fokienia hodginsii* and *Pinus massoniana*. *Wuyi Sci. J.* **2007**, *23*, 110–114.
54. Su, Z.W. Effects of different densities on understory plants and soil fertility of *Fokienia hodginsii. East China For. Manag.* **2007**, *4*, 26–28.
55. Ji, Z.J.; Quan, X.K.; Wang, C.K. Variations in leaf anatomy of *Larix gmelinii* reflect adaptation of its photosynthetic capacity to climate changes. *Acta Ecol. Sin.* **2013**, *33*, 6967–6974.
56. Ahrens, C.W.; Andrew, M.E.; Mazanec, R.A.; Ruthrof, K.X.; Challis, A.; Hardy, G.; Byrne, M.; Tissue, D.T.; Rymer, P.D. Plant functional traits differ in adaptability and are predicted to be differentially affected by climate change. *Ecol. Evol.* **2019**, *10*, 232–248.

57. Gougherty, A.V.; Keller, S.R.; Fitzpatrick, M.C. Maladaptation, migration and extirpation fuel climate change risk in a forest tree species. *Nat. Clim. Change* **2021**, *11*, 166–171.
58. Gao, M.; Xiong, C.; Gao, C.; Tsui, C.K.M.; Wang, M.M.; Zhou, X.; Zhang, A.M.; Cai, L. Disease-induced changes in plant microbiome assembly and functional adaptation. *Microbiome* **2021**, *9*, 187.
59. Rolfe, S.A.; Griffiths, J.; Ton, J. Crying out for help with root exudates: Adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. *Curr. Opin. Microbiol.* **2019**, *49*, 73–82.
60. Wen, T.; Zhao, M.; Liu, T.; Huang, Q.; Yuan, J.; Shen, Q. High abundance of *Ralstonia solanacearum* changed tomato rhizosphere microbiome and metabolome. *BMC Plant Biol.* **2020**, *20*, 166.
61. Dubey, A.; Malla, M.A.; Kumar, A. Taxonomical and functional bacterial community profiling in disease-resistant and disease-susceptible soybean provenances. *Braz. J. Microbiol.* **2022**, *53*, 1355–1370.
62. Singh, A.; Kumar, M.; Chakdar, H.; Pandiyan, K.; Kumar, S.C.; Zeyad, M.T.; Singh, B.N.; Ravikiran, K.T.; Mahto, A.; Srivastava, A.K.; et al. Influence of host genotype in establishing root associated microbiome of indica rice provenances for plant growth promotion. *Front. Microbiol.* **2022**, *13*, 1033158.
63. Li, Z.; Ma, L.; Zhang, Y.; Zhao, W.H.; Zhao, B.Z.; Zhang, J.B. Effect of wheat provenances with different resistance to *Fusarium* head blight on rhizosphere *Fusarium graminearum* abundance and microbial community composition. *Plant Soil* **2020**, *448*, 383–397.
64. Beschoren da Costa, P.; Benucci, G.M.N.; Chou, M.Y.; Van Wyk, J.; Chretien, M.; Bonito, G. Soil Origin and Plant Genotype Modulate Switchgrass Aboveground Productivity and Root Microbiome Assembly. *mBio* **2020**, *13*, e0007922.
65. Anderson, M.; Habiger, J. Characterization and identification of productivity-associated rhizobacteria in wheat. *Appl. Environ. Microbiol.* **2021**, *78*, 4434–4446.
66. Alahmad, A.; Edelman, L.; Castel, L.; Bernardon-Mery, A.; Laval, K.; Trinsoutrot-Gattin, I.; Thioye, B. Prebiotics: A Solution for Improving Plant Growth, Soil Health, and Carbon Sequestration. *J. Soil Sci. Plant Nutr.* **2023**, *23*, 6647–6669.
67. Wu, T.Y.; Wang, Y.H.; Wu, F.; Wu, X.Q. Dual inoculation with rhizosphere-promoting bacterium Bacillus cereus and beneficial fungus *Peniophora cinerea* improves salt stress tolerance and productivity in willow. *Microbiol. Res.* **2023**, *268*, 127280.
68. Guo, W.; Hao, H.; Zhang, W.H.; Hu, Z.H.; Leng, P.S. Ectomycorrhizal fungi enhance salt tolerance of *Quercus mongolica* by regulating ion balance. *J. Ecol.* **2022**, *33*, 3303–3311.
69. Mautner, M.N.; Conner, A.J.; Killham, K.; Deamer, D.W. Biological potential of extraterrestrial materials Microbial and plant responses to nutrients in the Murchison carbonaceous meteorite. *Icarus* **1997**, *129*, 245–253.
70. Jinal, H.N.; Gopi, K.; Prittesh, P.; Kartik, V.P.; Amaresan, N. Phytoextraction of iron from contaminated soils by inoculation of iron-tolerant plant growth-promoting bacteria in Brassica juncea *L. Czern. Environ. Sci. Pollut. Res.* **2019**, *26*, 32815–32823.
71. Sharma, P.; Chaturvedi, P.; Chandra, R.; Kumar, S. Identification of heavy metals tolerant *Brevundimonas* sp. from rhizospheric zone of *Saccharum munja* L. and their efficacy in in-situ phytoremediation. *Chemosphere* **2022**, *295*, 133823.
72. Chelius, M.K.; Triplett, E.W. The Diversity of Archaea and Bacteria in Association with the Roots of *Zea mays* L. *Microb. Ecol.* **2001**, *41*, 252–263.
73. Simon, H.M.; Dodsworth, J.A.; Goodman, R.M. Crenarchaeota colonize terrestrial plant roots. *Environ. Microbiol.* **2000**, *2*, 495–505.
74. Orellana, L.H.; Francis, T.B.; Ferraro, M.; Hehemann, J.H.; Fuchs, B.M.; Amann, R.I. Verrucomicrobiota are specialist consumers of sulfated methyl pentoses during diatom blooms. *ISME J.* **2022**, *16*, 630–641.
75. Sichert, A.; Corzett, C.H.; Schechter, M.S.; Unfried, F.; Markert, S.; Becher, D.; Fernandez-Guerra, A.; Liebeke, M.; Schweder, T.; Polz, M.F.; et al. Verrucomicrobia use hundreds of enzymes to digest the algal polysaccharide fucoidan. *Nat. Microbiol.* **2020**, *5*, 1026–1039.
76. He, S.; Stevens, S.L.R.; Chan, L.K.; Bertilsson, S.; Glavina Del Rio, T.; Tringe, S.G.; Malmstrom, R.R.; McMahon, K.D. Ecophysiology of Freshwater Verrucomicrobia Inferred from Metagenome-Assembled Genomes. *mSphere* **2017**, *2*, e00277-17.
77. Prathna, T.C. Significance of microbial volatiles in ecological health: Impact on wetland systems. In *Volatiles and Metabolites of Microbes*; Academic Press: Cambridge, MA, USA, 2021; pp. 163–176.
78. Cao, M.M.; Wang, F.; Zhou, B.H.; Chen, H.L.; Yuan, R.F. Community Distribution of the Rhizospheric and Endophytic Bacteria of *Phragmites australis* and Their Limiting Factors in Iron Tailings. *Huan Jing Ke Xue* **2021**, *42*, 4998–5009.
79. Li, Y.Y.; Pan, F.X.; Yao, H.Y. Response of symbiotic and asymbiotic nitrogen-fixing microorganisms to nitrogen fertilizer application. *J. Soils Sediments* **2019**, *19*, 1948–1958.
80. Fernandes, G.L.; Shenoy, B.D.; Damare, S.R. Diversity of bacterial community in the oxygen minimum zones of Arabian Sea and Bay of Bengal as deduced by Illumina sequencing. *Front. Microbiol.* **2020**, *10*, 3153.
81. Paingankar, M.S.; Ahire, K.; Mishra, P.; Rajpathak, S.; Deobagkar, D.D. Microbial diversity of the Arabian Sea in the oxygen minimum zones by metagenomics approach. *BioRxiv* **2019**, BioRxiv:731828.
82. Li, X.Y.; Duan, A.; Zhang, J.G. Site index for Chinese fir plantations varies with climatic and soil factors in southern China. *J. For. Res.* **2022**, *33*, 1765–1780.
83. Mao, L.; Zha, R.; Chen, S.; Zhang, J.; Jie, L.; Zha, X. Mixture Compound Fertilizer and Super Absorbent Polymer Application Significantly Promoted Growth and Increased Nutrient Levels in *Pinus massoniana* Seedlings and Soil in Seriously Eroded Degradation Region of Southern China. *Front. Plant Sci.* **2021**, *12*, 763175.
84. Tarin, M.W.K.; Fan, L.; Xie, D.; Tayyab, M.; Rong, J.; Chen, L.; Muneer, M.A.; Zheng, Y. Response of Soil Fungal Diversity and Community Composition to Varying Levels of Bamboo Biochar in Red Soils. *Microorganisms* **2021**, *9*, 1385.

85. Li, Y.Y.; Zhao, M.; Chen, W.W.; Du, H.Y.; Xie, X.D.; Wang, D.B.; Dai, Y.; Xia, Q.Y.; Wang, G.H. Comparative transcriptomic analysis reveals that multiple hormone signal transduction and carbohydrate metabolic pathways are affected by *Bacillus cereus* in *Nicotiana tabacum*. *Genomics* **2020**, *112*, 4254–4267.
86. Feng, J.; Rana, S.; Liu, Z.; Wang, Y.M.; Cai, Q.F.; Geng, X.D.; Zhou, H.N.; Zhang, T.; Wang, S.S.; Xue, X.Y.; et al. Diversity Analysis of Leaf Nutrient Endophytes and Metabolites in Dioecious *Idesia polycarpa* Maxim Leaves during Reproductive Stages. *Life* **2022**, *12*, 2041.
87. Li, F.; Zhang, X.M.; Gong, J.Y.; Liu, L.X.; Yi, Y. Specialized core bacteria associate with plants adapted to adverse environment with high calcium contents. *PLoS ONE* **2018**, *13*, e0194080.
88. Xiong, M.Y.; Jiang, W.; Zou, S.Z.; Kang, D.; Yan, X.C. Microbial carbohydrate-active enzymes influence soil carbon by regulating the of plant- and fungal-derived biomass decomposition in plateau peat wetlands under differing water conditions. *Front. Microbiol.* **2023**, *14*, 1266016.
89. Dong, N.Q.; Lin, H.X. Contribution of phenylpropanoid metabolism to plant development and plant-environment interactions. *J. Integr. Plant Biol.* **2021**, *63*, 180–209.
90. Yang, J.; Zhang, T.; Mao, H.; Jin, H.; Sun, Y.; Qi, Z. A Leymus chinensis histidine-rich Ca^{2+}-binding protein binds Ca^{2+}/Zn^{2+} and suppresses abscisic acid signaling in Arabidopsis. *J. Plant Physiol.* **2020**, *252*, 153–209.
91. Gu, S.; Yang, T.; Shao, Z.; Wang, T.; Cao, K.; Jousset, A.; Friman, VP.; Mallon, C.; Mei, X.; Wei, Z.; Xu, Y.; et al. Siderophore-Mediated Interactions Determine the Disease Suppressiveness of Microbial Consortia. *mSystems* **2020**, *5*, e00811-19.
92. Kim, S.C.; Wang, X. Phosphatidic acid: An emerging versatile class of cellular mediators. *Essays Biochem.* **2020**, *64*, 533–546.
93. Richard, C.T.; Elizabeth, A.H.; Pilon, S. Selenium transport and metabolism in plants: Phytoremediation and biofortification implications. *J. Hazard. Mater.* **2021**, *4*, 0304–3894.