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Responses of Rhizosphere Soil Chemical Properties and Bacterial Community Structure to Major Afforestation Tree Species in Xiong'an New Area

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Abstract: To explore the response of rhizosphere chemical and biological properties to eight major afforestation species in Xiong'an New Area, we measured rhizosphere soil properties in their pure stands and analyzed the bacterial community structure using a high-throughput sequencing platform. The results showed that: (1) Compared with coniferous species, broadleaved species had higher total nutrient concentration and pH in the rhizosphere but lower available nutrient concentration and soil moisture. Nitrate nitrogen deficiency was found in all stands. (2) *Uncultured_bacterium_f_Longimicrobiaceae* and *RB41* could distinguish *Platycladus orientalis* (Linn.) Franco from other trees. Compared with other tree species, *Sabina chinensis* (Linn.) Ant., *Armeniaca vulgaris* Lam., and *Fraxinus chinensis* Roxb. gathered more Actinobacteria, Planctomycetes, and Gemmatimonadetes, respectively. *Uncultured_bacterium_o_Rokubacteriales*, *uncultured_bacterium_f_Gemmatimonadaceae*, and *uncultured_bacterium_c_Subgroup_6* were major contributors to the differences in bacterial communities among most tree species. (3) Species characteristics changed soil chemical properties, further affecting the bacterial community. Total carbon, organic matter, total nitrogen, and pH were the main factors explaining these variations. In general, *Sophora japonica* Linn. and *F. chinensis* could increase soil total nutrient significantly, which meant that they were more suitable for afforestation in the studied area than the other species. *P. orientalis* and *Pinus tabuliformis* Carr. were better choices among conifers. We suggest planting more mixed forests to improve the rhizosphere nutrient status of conifers. A suitable way to alleviate prevailing nitrogen and phosphorus limitations is also required, such as introducing understory vegetation or supplementing organic fertilizers.

Keywords: plantation; tree species; rhizosphere; soil chemical properties; soil bacterial community

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1. Introduction

With economic and political development, a series of changes occur in land use and land cover that pose a great threat to biodiversity [1]. Forest degradation due to industrialization and urbanization is one of the most vital reasons behind these changes [2]. Forests play an irreplaceable role in the global carbon cycle with their large carbon pools [3]. However, human interference directly weakens their carbon neutrality ability, further aggravating global warming [4]. Therefore, people have begun to adopt various measures to alleviate forest decline since the last century [5]. Plantation refers to a forest predominantly composed of trees established through planting or deliberate seeding [6]. It can provide habitats for many species and restore forest ecosystems in a short time, especially in areas where natural forests are largely degraded [7,8]. However, after in-depth research, people gradually found that blind planting in pursuit of forest expansion may bring biodiversity decline or ecosystem resilience reduction [9]. Hence, scientifically based plantation is of great importance to maximize the ecological benefits [10].

Tree species selection is a key step in afforestation, as their characteristics largely determine the survival rate and growth of seedlings [11]. Evaluating the afforestation effect of different species through rhizosphere properties can reflect practical problems, because

there is a close relationship between trees and soil, while the rhizosphere is the area in which they directly interact [12]. Litter and exudates are the main sources of rhizosphere nutrients; correspondingly, rhizosphere nutrient status can be used as an indicator of tree characteristics and health [13]. Researchers often assess tree growth or nutrient utilization through soil total carbon, total nitrogen, and available nutrient concentration, as well as some physical properties [14–16]. Microorganisms, an important part of soil, are influenced by both soil and trees [17]. Tree species identity and genotypes directly contribute to the shaping of microbial communities [18]. They also affect microbial metabolic activity by influencing the soil environment [19]. Thus, the soil microbial community can accurately reflect the characteristics of the entire forest. Most previous studies about forest soil focused on fungi; however, more attention was paid to bacteria recently [20]. Scholars found that soil bacterial distribution is largely affected by nutrient availability, and some taxa mediate multiple key steps in element cycling [21]. It turned out that the in-depth exploration of bacterial composition and diversity was an effective means for studying soil development.

According to estimates, forests may offset 14.1% of national anthropogenic carbon emissions from 2010 to 2060, making a significant contribution to carbon neutrality [22]. Meanwhile, with the gradual narrowing of the carbon sink gap between natural forests and plantations, the carbon neutrality of plantations has received unprecedented attention [23]. Our research site, Xiong'an Millennium Forest, is located in North China, where the carbon sink of plantations is relatively high nationwide [24]. In order to explore the development model for regional forest ecosystems, the "Millennium Forest" project was launched in November 2017 [25]. The construction of this massive urban forestry system will continue until 2030, and the total forested area is expected to exceed 600 km^2 [26]. At present, more than 200 species of seedlings have been planted, so the timely evaluation of the afforestation effects of various species is the key to advance this project [27].

As a representative plantation in North China, Xiong'an Millennium Forest is of great value to carbon neutrality and is also a suitable site for exploring large-scale plantation management [28]. However, related research is still in its infancy, and many scientific questions remain to be addressed [29]. So, this study chose eight commonly used species in Millennium Forest, aiming to explore how tree species affected the rhizosphere chemical properties and bacterial community structure and further evaluate the afforestation effects of different stands. Our purposes were to provide reasonable suggestions for following afforestation and to offer new ideas for the selection of afforestation methods and tree species in North China.

2. Materials and Methods

2.1. Site Description and Experimental Design

Geographically, Xiong'an New Area is situated in Baoding City, on the eastern side of Taihang Mountain. The sampling site is located in Xiong County, which is part of Xiong'an New Area together with Rongcheng and Anxin County, and the total area is about 1770 km^2 (38°43′–39°10′ N, 115°38′–116°20′ E) [30] (Figure 1). The studied area has a warm temperate monsoon continental climate. The main soil type here is fluvo-aquic soil, as well as some salinized fluvo-aquic and fluvo-aquic cinnamon soil [31]. Average annual temperature, precipitation, and frost-free period are 12.4 °C, 495.1 mm, and 204 d, respectively [32].

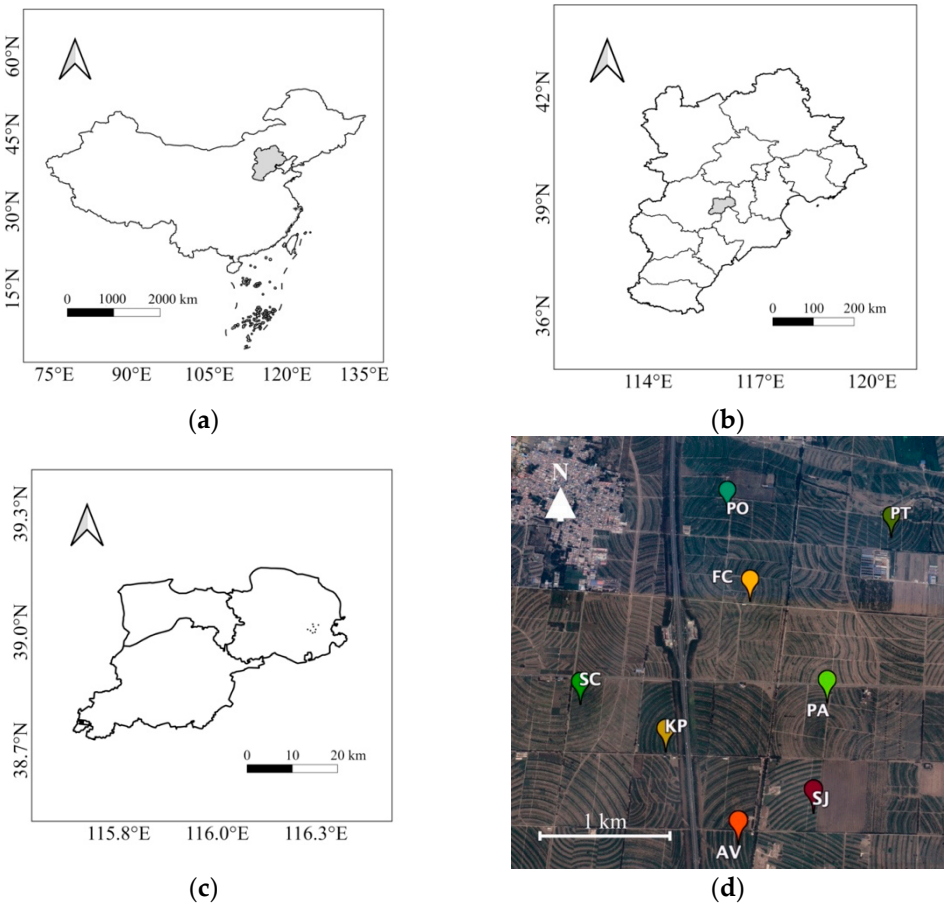


Figure 1. Geographical location of sampling site: (**a**) China; (**b**) Beijing–Tianjin–Hebei urban agglomeration; (**c**) Xiong'an New Area; (**d**) studied stands (http://earth.google.com/ (accessed on 10 October 2022)). (AV, *A. vulgaris*; SJ, *S. japonica*; KP, *K. paniculata*; FC, *F. chinensis*; PO, *P. orientalis*; PT, *P. tabuliformis*; PA, *P. asperata*; SC, *S. chinensis*.)

Since the launch of the "Millennium Forest" project, over 285.3 km^2 of forest has been planted [33]. We selected *Armeniaca vulgaris* Lam., *Sophora japonica* Linn., *Koelreuteria paniculata* Laxm., *Fraxinus chinensis* Roxb., *Platycladus orientalis* (Linn.) Franco, *Pinus tabuliformis* Carr., *Picea asperata* Mast., and *Sabina chinensis* (Linn.) Ant. after a field investigation. Species selection demonstrated the collocation principles of evergreen coniferous and deciduous broadleaved species, fast- and slow-growing species, and shade-tolerant and -intolerant species for this project [34]. Samples were collected from eight pure stands planted in spring 2018 (composed of the eight species mentioned above), whose planting spacing was 1.5–2 m × 2 m in all cases; their basic information is shown in Tables 1 and S1. All stands were managed in a "close-to-nature" manner, with no thinning or fertilizer application, aimed to achieve self-regulation and self-renewal. Samples from different stands were used for soil properties determination and bacterial community analyses. On this basis, we compared the measured indicators to explore the impact of the tree species.

Table 1. Basic characteristics of studied plantations.

Tree Species Composition	Area (ha)	Density (Stems·ha^{-1})	Location
Armeniaca vulgaris	0.83	1963	38.997856 N, 116.241925 E
Sophora japonica	0.91	1930	38.999703 N, 116.248072 E
Koelreuteria paniculata	1.30	1873	39.003558 N, 116.236375 E
Fraxinus chinensis	0.85	1908	39.013431 N, 116.243931 E
Platycladus orientalis	0.82	1824	39.019797 N, 116.242372 E
Pinus tabuliformis	1.35	1927	39.017867 N, 116.256533 E
Picea asperata	0.96	1897	39.006622 N, 116.249847 E
Sabina chinensis	0.65	1912	39.006608 N, 116.229425 E

2.2. Soil Sampling Method

Soil samples were collected in July 2021. Three sample plots (100 m^2 each) were set up in the middle and diagonal of eight pure stands, and basic information such as height and diameter at breast height were recorded. In order to ensure representativeness, we chose five trees in each plot following an S-shaped line randomly [35]. We started at the base of the roots and dug outwards to find fine roots in the 0–20 cm soil layer, and rhizosphere soil was obtained by shaking off soil adhering to fine roots, taking care to remove larger clods [36]. Soil collected from the same plot was thoroughly mixed and then divided into two parts: one was stored at −80 °C for high-throughput sequencing at Biomarker Technologies (Beijing) Co., Ltd., (Beijing, China) and the other was dried naturally, ground, and sieved through a 60-mesh sieve for chemical property determination.

2.3. Soil Chemical Analysis

We used a Vario MICRO Cube elemental analyzer (Elementar, Langenselbold, Germany) to determine total nitrogen (TN) and total carbon (TC); then, we applied molybdenum antimony spectrophotometry to measure total phosphorus (TP). The determination method of organic matter (OM) was potassium dichromate oxidation spectrophotometry [35,37]. Potassium chloride solution extraction spectrophotometry was used for the determination of ammonium nitrogen (NH_{4^+}-N) and ultraviolet spectrophotometry for nitrate nitrogen (NO_{3^-}-N). Molybdenum antimony anti-color spectrophotometry was selected to measure available phosphorus (AP) [38–40]. We determined soil pH using the 2.5:1 water–soil ratio electrode method and then determined soil moisture content (SM) using the gravimetric method [41,42].

2.4. Bioinformatic Analysis

TGuide S96 Magnetic Bead Method Soil Genomic DNA Extraction Kit (Tiangen Biotech (Beijing) Co., Ltd. (Beijing, China)) was applied to extract nucleic acid. Then, we amplified the bacterial 16S V3 + V4 region with primer sequences 338F (5′-ACTCCTACGGGAG GCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) [43]. A 10 μL PCR system was used including 5–50 ng of genomic DNA, forward and reverse primers (0.3 μL and 10 μM, respectively), 5 μL of KOD FX Neo Buffer, 2 μL of dNTP (2 mM each), and 0.2 μL of KOD FX Neo. The reaction consisted of 25 cycles of initial denaturation at 95 °C for 60 s, 95 °C for 30 s, 50 °C for 30 s, 72 °C for 40 s, and extended at 72 °C for 7 min. A Monarch DNA gel recovery kit was applied for gel cutting and recovery after electrophoresis and purification. Biomarker Technologies (Beijing) Co., Ltd., constructed a library and sequenced on the Illumina Novaseq 6000 platform. We used Trimmomatic (ver. 0.33) to filter raw dates and Cutadapt (ver. 1.9.1) to identify and remove primer sequences. Through Usearch (ver. 10), clean reads were spliced via overlapping, followed by length filtering. Chimera sequences were then removed using UCHIME (ver. 4.2). We obtained OTUs with Usearch, which clustered effective reads at a similarity level of 97.0%. Classification was based on the SILVA database (Release128; http://www.arb-silva.de, accessed on 20 May 2022), and QIIME2 (https://qiime2.org/, accessed on 31 May 2022) was used to calculate α-diversity.

2.5. Statistical Analysis

The one-way ANOVA in SPSS 26.0 was used to compare the differences in soil properties, the relative abundance of bacterial taxa, and α-diversity indices among different tree species; the results were plotted using Prism 9.4.0. For the bacterial community, we used NMDS (using Canoco 5.0), PERMANOVA (using Vegan Package 2.6–4 of Rstudio 2022.07.2 + 576), and SIMPER (using Past 4.11) to demonstrate their structural differences among eight tree species while further searching for key taxa leading to significant differences. For LefSe analyses, we conducted the non-parametric factorial Kruskal–Wallis rank sum test to detect the species with significant differences in abundance among different groups. Then, the Wilcoxon rank sum test was used to conduct a pairwise test on the subgroups in each group. A linear discriminant analysis (LDA) was applied for data dimension reduction and to find biomarkers with statistical differences among tree species (using BMKCloud (www.biocloud.net, accessed on 30 March 2022)) [44]. An RDA was conducted using Canoco 5.0 to illustrate the correlations between soil chemical properties and bacterial communities. A Bioenv analysis (using the Vegan package of Rstudio) revealed the environmental variable subset most associated with community differences. The same package was used to perform a variation partitioning analysis, explaining the contribution of species characteristics and soil properties to microbial community variations.

3. Results

3.1. Soil Chemical Properties

The rhizosphere chemical properties of the studied species showed significant differences ($p < 0.05$) (Figure 2, Table S2). TN and TC concentrations in *S. japonica* rhizosphere were the highest, while *K. paniculata* and *F. chinensis* had more TP and OM than other species, respectively. The lowest values of the above four factors all appeared in *S. chinensis* rhizosphere; *P. orientalis* and *P. asperata* were also at significantly lower levels ($p < 0.05$). The available nutrient concentrations in coniferous species rhizosphere were generally higher than in those of broadleaved species. *P. asperata* had the highest C:N and C:P; *S. chinensis* had the highest N:P and the lowest C:N; C:P and N:P were the lowest in *K. paniculata* rhizosphere. Broadleaf species had lower soil moisture and higher pH than conifers.

3.2. Bacterial Community Composition

Bacteria from 25 phyla and 388 genera were detected in all samples, and the top 10 taxa in relative abundance are shown in Figure 3 and Tables S3 and S4. The relative abundance of Acidobacteria in *S. japonica* rhizosphere was significantly higher than that in *P. asperata*, while *P. asperata* had more Proteobacteria than *S. japonica*, *F. chinensis*, *P. orientalis*, and *K. paniculate* ($p < 0.05$). *F. chinensis* and *S. chinensis* had the highest and lowest abundances of Gemmatimonadetes, respectively, but both obviously enriched the growth of Actinobacteria more ($p < 0.05$). *A. vulgaris* rhizosphere contained the least amount of Actinobacteria, while Nitrospirae showed a roughly opposite pattern to Actinobacteria. Rokubacteria and Planctomycetes in the rhizospheres of *A. vulgaris*, *P. orientalis*, *P. tabuliformis*, and *K. paniculata* were significantly higher than those in *S. chinensis*. *P. asperata* rhizosphere had visibly more Bacteroidetes than *P. orientalis* ($p < 0.05$).

Except for *P. orientali* and *P. tabuliform*, *uncultured_bacterium_c_Subgroup_6* in the rhizosphere of *S. japonica* was the most abundant. *P. orientali* significantly enriched the growth of *RB41* more than the other species, apart from *S. japonica* and *S. chinensis* ($p < 0.05$). *Sphingomonas* showed higher abundance in *S. chinensis* rhizosphere than in *A. vulgaris* and *S. japonica*. *Uncultured_bacterium_f_Geminicoccaceae* was the least abundant in *F. chinensis* rhizosphere, but *F. chinensis* had obviously more *Bryobacter* than *A. vulgaris* and *P. asperata* ($p < 0.05$). Other dominant genera showed the lowest abundance in *S. chinensis* rhizosphere, among which *uncultured_bacterium_f_Gemmatimonadaceae* and *MND1* were enriched in *F. chinensis* rhizosphere, while *uncultured_bacterium_o_Rokubacteriales* and *Nitrospira* gathered more in *A. vulgaris* rhizosphere.

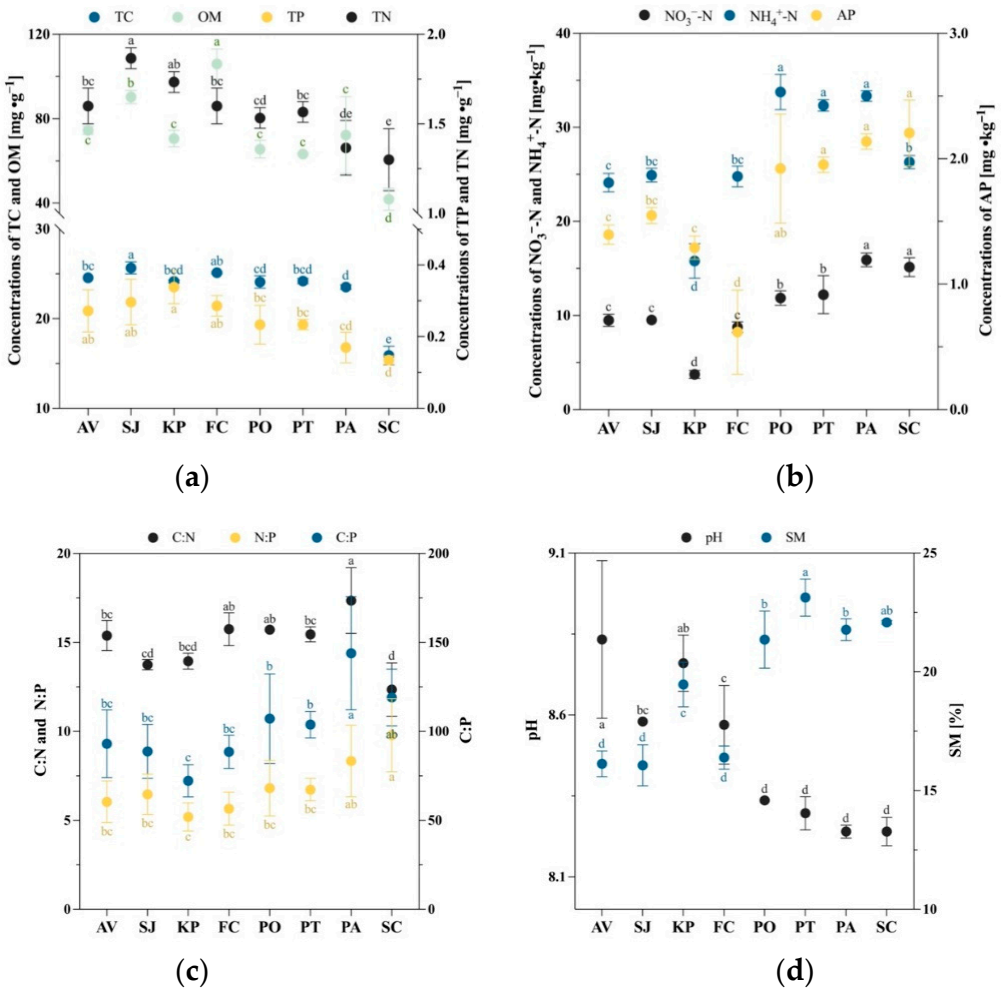


Figure 2. Rhizosphere chemical properties in different stands. Data present means ± st. errors; different letters (from a to d) in figure body indicate significant differences among tree species after Duncan's test ($p < 0.05$), $n = 3$. (**a**) TC, TN, TP, and OM. (**b**) NH_4^+-N, NO_3^--N, and AP. (**c**) C:N, C:P and N:P. (**d**) pH and SM. (TN, total nitrogen; TC, total carbon; TP, total phosphorus; OM, soil organic matter; NO_3^--N, nitrate nitrogen; NH_4^+-N, ammonium nitrogen; AP, available phosphorus; SM, soil moisture content. AV·, *A. vulgaris*; SJ, *S. japonica*; KP, *K. paniculata*; FC, *F. chinensis*; PO, *P. orientalis*; PT, *P. tabuliformis*; PA, *P. asperata*; SC, *S. chinensis*).

3.3. Biomarkers

From phylum to species, 27 biomarkers were filtered out, and their relative abundances were significantly higher for particular tree species; thus, they could be representative indicators ($p < 0.05$) (Figure 4). Taxa from Proteobacteria were biomarkers of multiple tree species, such as Sphingomonadaceae, Deltaproteobacteria, and Geminicoccaceae, and *MND1* was biomarker for *S. chinensis*, *P. orientali*, *P. tabuliform*, and *F. chinensis*, respectively. In addition, Acidimicrobiia was enriched in *S. chinensis* rhizosphere, and *RB41* was enriched in *P. orientali* rhizosphere; taxa from Gemmatimonadaceae were enriched in F. chinensis rhizosphere. *A. vulgaris* was characterized by a higher abundance of Planctomycetes as well as taxa from NC10.

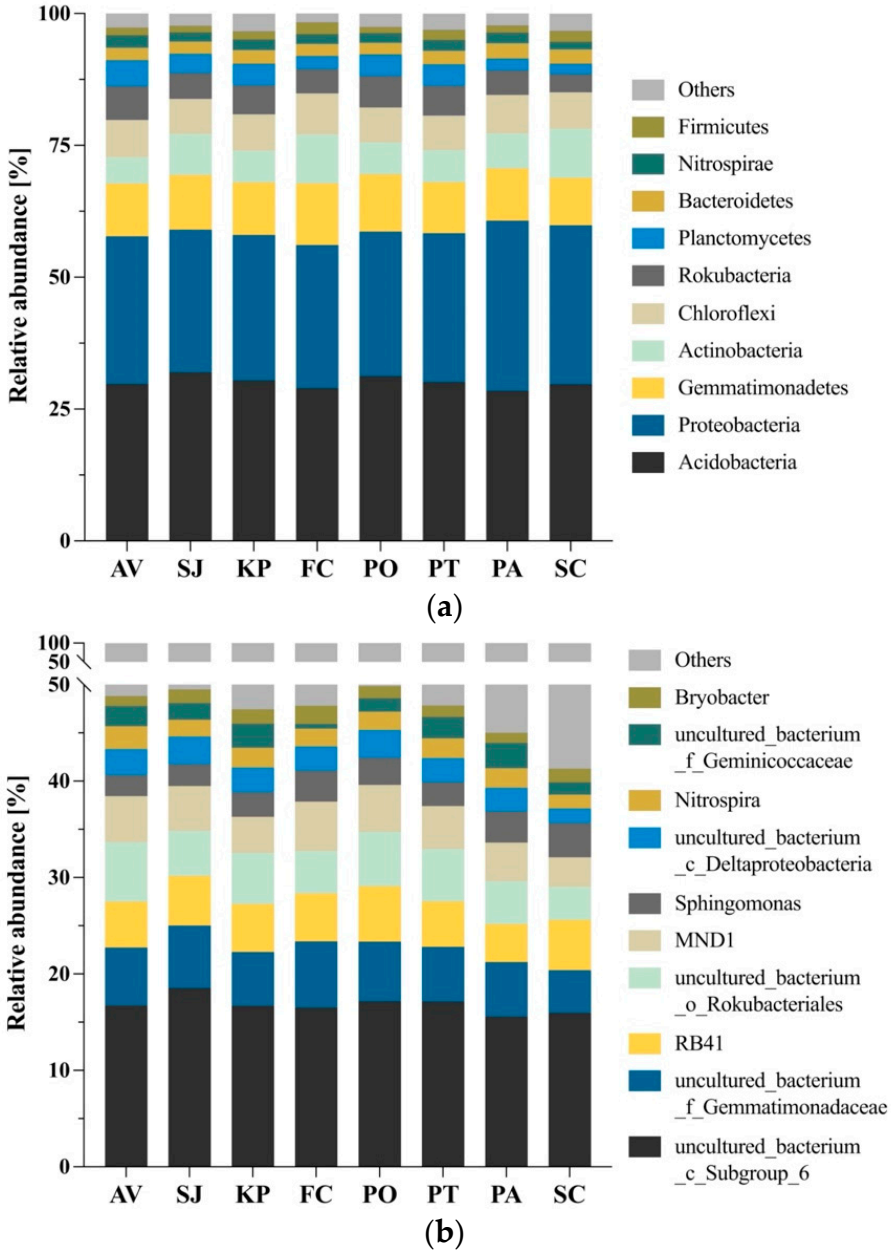


Figure 3. Rhizosphere bacterial abundance in different stands: (**a**) at the phylum level; (**b**) at the genus level. (AV, *A. vulgaris*; SJ, *S. japonica*; KP, *K. paniculata*; FC, *F. chinensis*; PO, *P. orientalis*; PT, *P. tabuliformis*; PA, *P. asperata*; SC, *S. chinensis*).

3.4. α-Diversity

The Shannon indices of *P. asperata* and *S. chinensis* rhizosphere were significantly higher than those of *A. vulgaris*, *K. paniculata*, and *P. orientali*, while *P. asperata* rhizosphere had a higher Simpson index than other species, except for *S. chinensis* ($p < 0.05$). Broadleaved species generally showed lower Shannon and Simpson indices than conifers. *S. chinensis* rhizosphere had the lowest values of the other four indices, and most of the differences were significant; additionally, these indices of *F. chinensis* were also at low levels, and differences among the other six species were not significant ($p < 0.05$) (Figure 5, Table S5).

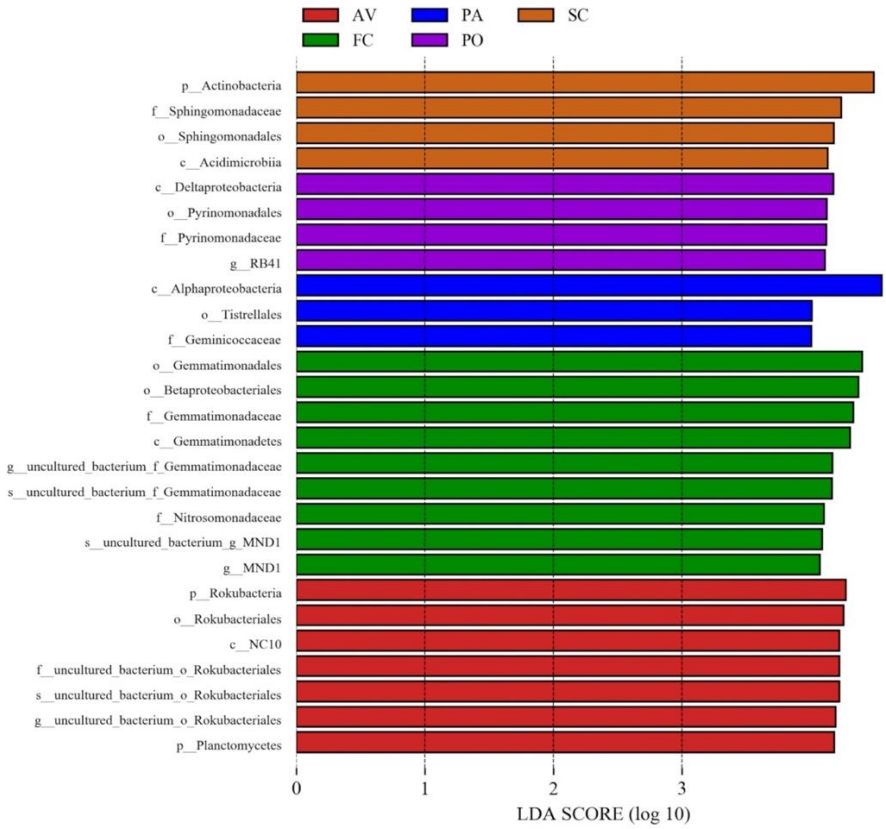


Figure 4. LEfSe analyses of rhizosphere bacterial taxa. The figure shows taxa whose LDA scores were greater than the set value of 4.0, and the length of the histogram represents the impact of different species.

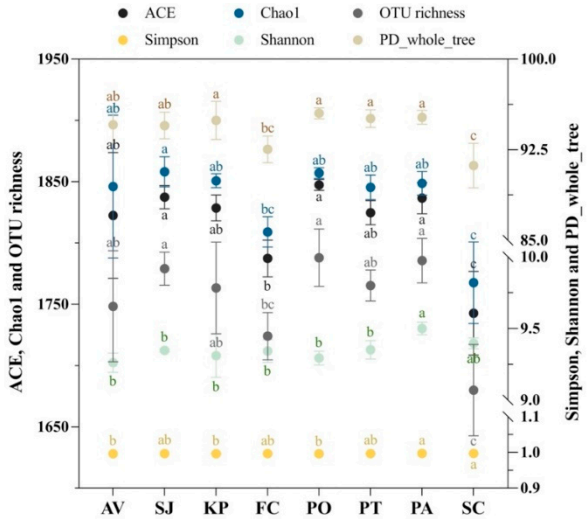


Figure 5. Rhizosphere bacterial community α-diversity in different stands. Data present means ± st. errors; different letters (from a to d) in figure body indicate significant differences among tree species after Duncan's test ($p < 0.05$), $n = 3$. (AV, *A. vulgaris*; SJ, *S. japonica*; KP, *K. paniculata*; FC, *F. chinensis*; PO, *P. orientalis*; PT, *P. tabuliformis*; PA, *P. asperata*; SC, *S. chinensis*).

3.5. β-Diversity

The rhizosphere bacterial composition was significantly different among most species studied ($p < 0.05$) (Figure 6, Table S6). The bacterial composition of *S. chinensis* was obviously different from that of other species, as was that of *F. chinensis*. *P. asperata* had

no heterogeneity only with *P. tabuliform*. There were also significant differences among *S. japonica*, *K. paniculata*, and *P. tabuliform* ($p < 0.05$).

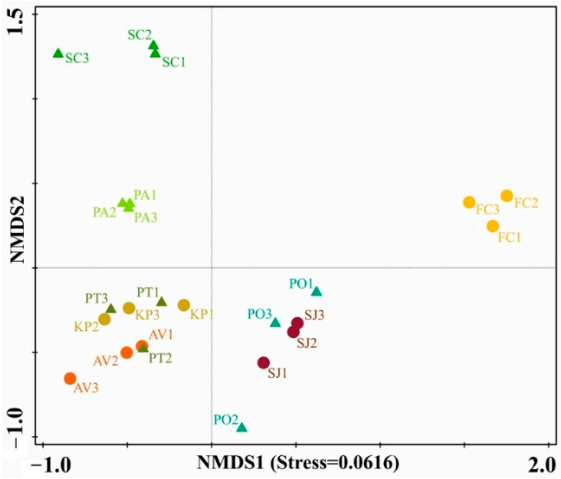


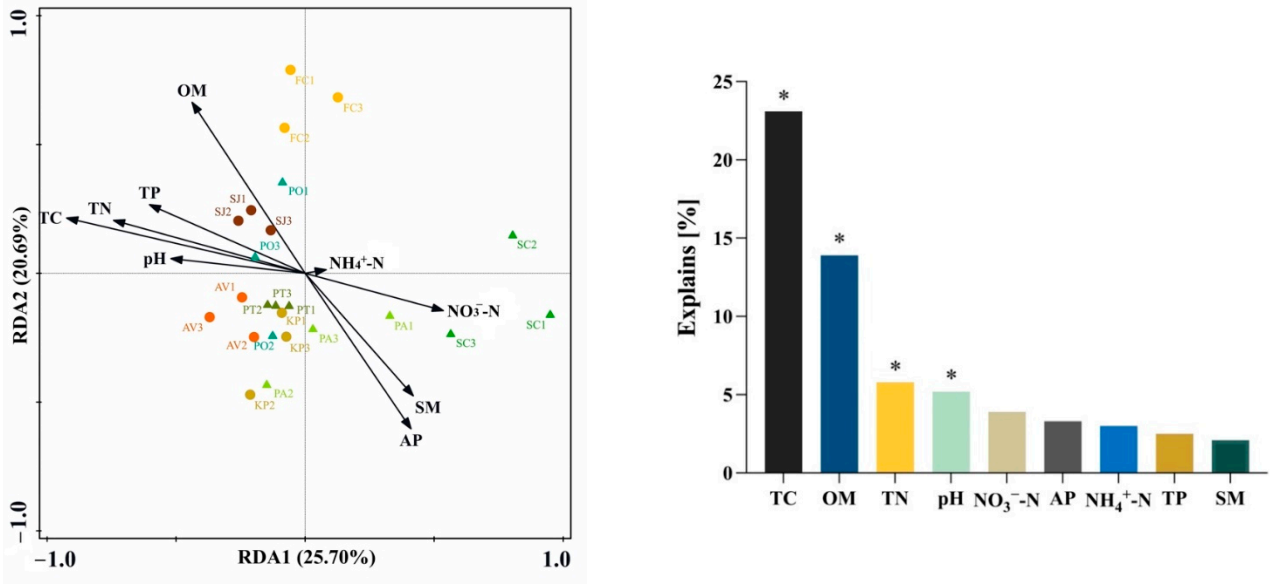
Figure 6. Nonmetric multidimensional scaling (NMDS) of rhizosphere bacterial community in different stands. (AV, *A. vulgaris*; SJ, *S. japonica*; KP, *K. paniculata*; FC, *F. chinensis*; PO, *P. orientalis*; PT, *P. tabuliformis*; PA, *P. asperata*; SC, *S. chinensis*).

The biomarkers of different tree species shown above were key contributors to the differences in the bacterial community structure due to their high abundance. However, the appearance of structural differences was often a combined effect of multiple taxa. It turned out that *uncultured_bacterium_f_Gemmatimonada*, *uncultured_bacterium_o_Rokubacteriales*, and *uncultured_bacterium_c_Subgroup_6* contributed significantly to bacterial composition differences among most species. *MND1* could distinguish *S. chinensis* from most species. *Uncultured_bacterium_f_Geminicoccaceae* and *RB41* played a leading role in the differences between *F. chinensis* and *P. tabuliform*, and other species, respectively. *AKYG587* was a key group for *A. vulgaris*, *K. paniculata*, and *P. tabuliform* to form unique bacterial community structures. *Sphiningomonas* had a certain contribution in comparison with *P. orientali* with other species, as well as *A. vulgaris* with conifers. *Uncultured_bacterium_f_Longimicrobiaceae* was an important discriminating genus of *P. orientali*, and it rarely showed a high contribution to the differences among other species (Table S7).

3.6. Driving Factors of Bacterial Community Structure

Except for OM, NH_4^+-N, C:P, and SM, the other soil properties had significant effects on bacterial α-diversity ($p < 0.05$). Among them, TN, TP, and pH were negatively correlated with the Shannon and Simpson indices, while NO_3^--N and AP showed positive effects. The other four indices were positively correlated with TC, TP, and C:N, while they were negatively correlated with N:P (Table S8).

We excluded C:N, C:P, and N:P after the collinear analyses, ensuring all remaining factors had a variance inflation factor of less than 10. The first axes explained 25.70% of the community structural variations, and the second axis explained 20.69%. Selected factors explained 62.80% of variation totally (Figure 7). The impacts of TC ($F = 6.6$, $p = 0.002$), OM ($F = 4.6$, $p = 0.005$), TN ($F = 2.1$, $p = 0.008$), and pH ($F = 1.8$, $p = 0.018$) reached the significant level. The environmental variable subset with the best correlation with community data included TC, OM, and AP (correlation of 0.76). The superposition effect of soil properties and tree species explained 68% of the bacterial community structure variations. The soil properties were the main reasons affecting bacteria, with an individual contribution rate of 47%, while that of tree species was 12%. Their interaction contribution reached 9.0% (Figure 8).



(**a**) (**b**)

Figure 7. (**a**) Redundancy analyses (RDAs) between soil properties and rhizosphere bacterial community in different stands. (**b**) Explanation rate of soil properties to bacterial community variation. * indicates significant differences ($p < 0.05$). (TN, total nitrogen; TC, total carbon; TP, total phosphorus; OM, soil organic matter; NO_3^--N, nitrate nitrogen; NH_4^+-N, ammonium nitrogen; AP, available phosphorus; SM, soil moisture content).

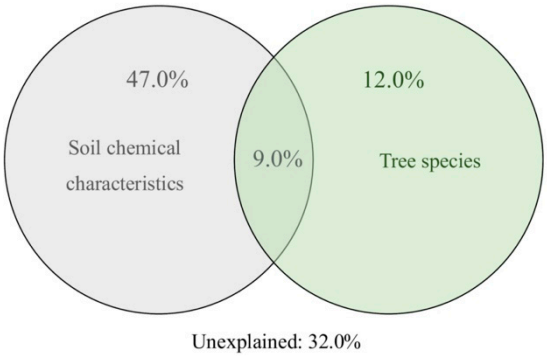


Figure 8. Variation partitioning analysis (VPA) between environmental variables and rhizosphere bacterial genera.

4. Discussion

4.1. Influences of Tree Species on Rhizosphere Soil Chemical Properties

Litter decomposition affects nutrient cycling remarkably, suggesting that various species might shape different soil properties, and our results were consistent with this [45]. Broadleaved trees usually have stronger photosynthetic capacity, as they assimilate and store more carbon [46]; the carbon concentration in exudates is also positively correlated with carbon allocated to roots, and such characteristics have a considerable impact on soil carbon inputs between broadleaved and coniferous species [47]. Due to the shorter leaf lifespan, deciduous broadleaved trees compensate for carbon uptake by producing leaves containing more nitrogen and phosphorus, while the needles of evergreen conifers have more lignin and tannin, with higher C:N and C:P [48,49]. These all make coniferous forests have a slower decomposition rate and less nutrient return and explain the generally higher total nutrient contents in broadleaved species rhizospheres [50]. The litter and exudates of conifers were shown to release more organic acids during decomposition, which also explains their lower rhizosphere pH [51]. Soil acidification was proved to be related to the excessive application of nitrogen fertilizers [52]. With the extension of afforestation

years, organic acids released by root exudation and litter decomposition also make soil acidification aggravated [53]. Regarding the studied stands located in the Beijing–Tianjin–Hebei region with severe soil salinization, their afforestation period was short, and no nitrogen fertilizer was applied after afforestation. We thought this might have been a reason why all studied stands maintained high pH values and low available nitrogen concentrations, compared with other research studies.

S. japonica had higher TC and TN in the rhizosphere, which was consistent with the conclusion that *S. japonica* is the most suitable to improve salinized soil [54]. *F. chinensis* also played an obvious role in increasing salinized soil OM, and this might be attributed to its rapid litter decomposition and better humification [55]. *K. paniculata* was confirmed to have the most soil TP during abandoned mine restoration; combined with our results, we believe it contributes to TP accumulation [56]. Though not completely significant, total nutrients in *P. tabuliform* rhizosphere were at a high level in conifers, but soil polarization caused by monoculture made it lack advantages compared with broadleaved species [57,58]. In our previous study, TC, TN, TP, and OM in *S. chinensis* rhizosphere increased obviously when mixed with broadleaved species [27]. *S. chinensis* might be affected by more substances that are difficult to decompose in its litter, which hinders the nutrient cycles [59].

Most evergreen species have conservative resource trade-off strategies, while deciduous species are on the acquisitive side [60]. Therefore, deciduous broadleaved species have lower available nutrient reserves in the rhizosphere, precisely because their consumption rate is greater than the mineralization rate [61]. Nitrogen limitation has a major impact on forest productivity, especially in the early stages of afforestation [62]. All samples had less NO_{3^-}-N than NH_{4^+}-N, which might have reflected that the nitrate nitrogen supply in the study area could no longer meet the needs of the trees. C:N and N:P proved our prediction. All C:N values were higher than the average Chinese level; a N:P value of less than 14 indicates nitrogen limitation, so all species were nitrogen-limited in this study [63,64], while *S. chinensis* had the lowest C:N, presumably because as a low-resource plant, it did not require much nitrogen supply [65]. The higher C:P of conifers indicated that their phosphorus utilization was also severely limited. Resource acquisition strategies also affect soil moisture content. Broadleaved species tend to absorb more water to meet growth needs [66]. However, needles that decompose slowly might form litter mats, and this slows down the water infiltration rate [16]. Interestingly, we found that the NH_{4^+}-N concentration was significantly correlated with SM. Through previous studies, we know that when soil moisture and temperature are both low, the mineralization rate slows down, resulting in a decrease in the NH_{4^+}-N concentration [67].

As we could see, nutrient limitation was common in the study stands, especially in conifers. We suggest considering mixed forests containing both coniferous and broadleaved species in subsequent afforestation [68]. For broadleaved species with higher requirements, organic fertilizer could be applied [69]. Introducing understory vegetation as green manure might also improve soil nutrients [70].

4.2. Dominant Taxa in Studied Stands

Acidobacteria were negatively correlated with the carbon mineralization rate and available nutrient content [71], while Proteobacteria and Bacteroidetes had quick growth responses with sufficient water and available nutrients [72]. Thus, *S. japonica* rhizosphere contained less available nutrients and had more Acidobacteria, but Proteobacteria and Bacteroidetes were enriched in *P. asperata* and *S. chinensis* rhizospheres. Broadleaved species rhizospheres generally had a higher abundance of Gemmatimonadetes, consistently with Wu's results, which indicated that Gemmatimonadetes were inversely proportional to available nitrogen [73]. The ability to solubilize complex carbon substrates caused an increase in the presence of Actinobacteria in *P. asperata* and *S. chinensis* rhizospheres [74]. Actinobacteria also play a role in cellulose decomposition; thus, we speculated that the enrichment in *F. chinensis* and *S. japonica* rhizospheres was possibly due to their large litter and high TC concentration [75]. NC10 participate in anaerobic methane oxidation, and

Planctomycetes contain anammox bacteria that control the rate-limiting step of nitrification; both require NO_2^- as electron acceptors [76–78]. Their enrichment might have indicated a higher NO_2^- concentration in *K. paniculata*, *P. orientali*, *P. tabuliform*, and especially *A. vulgaris* rhizospheres or more frequent anaerobic reactions. Interestingly, the other four species with less Planctomycetes were all commonly used in saline–alkali land, and we think that their distribution was related to tree salinity and alkali tolerance [75].

According to the above analysis, *S. japonica* might have been restricted by carbon availability, and the ability to promote OM decomposition was the key to the enrichment of *uncultured_bacterium_c_Subgroup_6* [79]. The increase in Gemmatimonadetes was closely related to soil environmental quality improvement, proving that despite the fact that all were conifers, *S. chinensis* rhizosphere environment was poorer [80]. Taxa from Gemmatimonadetes are also able to weather minerals and dissolve phosphate; their enrichment in *F. chinensis* and *S. japonica* rhizospheres might have motivated AP supply [58]. *MND1* form a group involved in nitrogen fixation and phosphate mineralization [81]; *Bryobacter* could promote carbon cycling and are positively correlated with soil enzyme activity [82]. Similar to the above speculation, we believe that this was a strategy adopted by *F. chinensis*; that is, it enhanced nutrient circulation by gathering various such taxa. Both *RB41* and *Sphiningomonas* are related to IAA secretion and are crucial in enhancing disease resistance [83,84]. They were biomarkers for *P. orientali* and *S. chinensis*, respectively; conifers might regulate growth through aggregating these taxa. Deltaproteobacteria were also a biomarker of *P. orientali*, showing a facilitating impact on pollutant degradation [85]. Therefore, *P. orientali* also shaped a better rhizosphere environment by reducing harmful substances in the soil. Similar to Deltaproteobacteria, Alphaproteobacteria participate in degradation, and both contain genes for nitrogen fixation [21]. Though functionally similar, they enrich in different rhizospheres. This is because the organic substances that could be utilized by each taxa of Proteobacteria are not exactly the same, and they respond diversely to different soil conditions for better environmental adaptation [86].

4.3. Effects of Tree Species on Soil Bacterial Diversity and Community Structure

For promoting nutrient cycling, trees gather related functional taxa in the rhizosphere. However, taxon composition changes according to tree physiological characteristics and soil conditions, further leading to structural variations in the entire community [87]. Soil carbon, nitrogen, and organic matter are considered to be critical for bacterial diversity, as they provide nutrients and energy while stimulating the growth of specific groups [88]. We think that significantly lower TC, TN, TP, and OM were the main reason for lower bacterial species richness in *S. chinensis* rhizosphere [89]. Defensive compounds that inhibit microbial activity, such as more lignin or polyphenols released through exudates, are also a possible reason [21,90]. Based on previous studies, we discovered that *F. chinensis* was more seriously damaged by *Hyphantria cunea* than other species in the study area [34]. The rhizosphere bacterium diversity of trees with poor health tends to decline, and taxon composition often changes correspondingly; this might explain the observed phenomenon [29]. Different taxa compete for limited nutrients, leading to the abundance of each group to vary and to the decrease in uniformity, while only excess required for microbial growth is released into the soil [91,92]. The more available nutrients in *S. chinensis* and *P. asperata* rhizospheres might have indicated that the competition for nutrients was not fierce and nutrient utilization was lower, which explained their higher community uniformity.

Taxa with a high contribution rate to bacterial structural differences were the dominant taxa in this study. They varied sensitively under different soil conditions [93]. However, some taxa, although relatively low in abundance, are integral to the construction of distinct bacterial community [94]. For example, *AKYG587*, which contributed evidently to differences among *A. vulgaris*, *K. paniculata*, *P. tabuliform*, and other species, is associated with disease suppression [95]. Taxa from Longimicrobiaceae have putative biosynthetic gene clusters of ribosomally synthesized and post-translationally modified peptides [96]. Their abundance decreases prominently under enhanced UV radiation and reduced rainfall [97].

P. orientali saplings are relatively shade tolerant, and higher soil moisture and the shaded environment created by their canopy might provide a suitable condition for this group [98].

4.4. Interplay among Trees, Soil Chemical Properties, and Bacterial Community

Trees are the main primary producers in forests, regulating the biogeochemical cycle and altering organic matter composition through their vital activity [20]. OM not only contains various nutrients required by trees but is also an energy source for bacterial reproduction, and its dynamics have a remarkable impact on bacterial communities [99]. TC and TN are considered as pivotal contributors to community structural differences, and both take part in bacterium individual formation while affecting their biomass [100,101]. As nutrient providers, their contents profoundly alter the taxon composition and metabolic activity, especially under nitrogen limitation, as in this study [78]. Beyond that, the responses of different taxa to soil pH are quite discrepant; the role of pH in vital ecological processes such as mineralization and humification cannot be ignored [102]. AP is the part that can be directly used. When nutrients are limited, microbes and trees compete for available nutrients; thus, their contents act on the community structure up to a point [103].

Tree characteristics largely determine the rhizosphere environment, while soil conditions have an intimate relation with the unique bacterial community structure of each tree species. This serves to show that trees affect rhizosphere bacteria by driving changes in soil properties [104]. The chemical properties of soil, as the immediate living environment of rhizosphere microbes, explain bacterial diversity and composition the most [105]. While the shaping of soil properties by trees activities only accounts for one part, climate and soil parent materials are all factors should be pondered [106,107]. Similarly, trees do not only affect the factors studied, as soil aggregate structure, animal activity, etc., also vary with different species [108,109].

5. Conclusions

The major tree species in Xiong'an New Area had significant impacts on rhizosphere properties, and they could shape the bacterial community structure by changing soil nutrient contents. In general, broadleaved species had more total carbon, total nitrogen, total phosphorus, and organic matter in the rhizosphere than conifers, with lower soil acidity; however, their higher requirements also led to a pronounced shortage of available nutrients. *P. orientalis* was distinguished from other species by the presence of *uncultured_bacterium_f_Longimicrobiaceae* and the significant enrichment of *RB41*. *S. chinensis*, *A. vulgaris*, and *F. chinensis* gathered more taxa from Actinobacteria, Planctomycetes, and Gemmatimonadetes, respectively. Dominant genera *uncultured_bacterium_c_Subgroup_6*, *uncultured_bacterium_f_Gemmatimonadaceae*, and *uncultured_bacterium_o_Rokubacteriales* were the main contributors to bacterial community differences.

The pure stands studied were generally limited in terms of nitrogen and phosphorus, especially conifers. Methods that provide nutrients to pure stands should be applied, such as introducing understory vegetation or mixing conifers with broadleaved species. In following afforestation projects, we suggest that broadleaved species such as *S. japonica* and *F. chinensis* are given priority, while for conifers, *P. orientalis* and *P. tabuliformis* are more suitable. By promoting a positive interaction among trees, soil nutrients, and bacterial community, the soil quality and tree growing conditions in Xiong'an New Area could be further improved.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13111822/s1, Table S1: Growth of studied species, Table S2: Rhizosphere physiochemical properties in different stands, Table S3: Top 10 phyla in relative abundance (%) of rhizosphere soil, Table S4: Top 10 genera in relative abundance (%) of rhizosphere soil, Table S5: α-diversity indices of rhizosphere bacterial community, Table S6: PERMANOVA of rhizosphere bacterial community; Table S7: SIMPER (similarity of percentage analysis) of rhizosphere bacterial community structure, Table S8: Pearson correlation analysis between soil physiochemical properties and bacterial community α-diversity.

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