

Article

Structure and Function of Rhizosphere Bacterial Communities in the Endangered Plant *Abies ziyuanensis*

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Abstract

Rhizosphere microbiota are key drivers of plant nutrition, immunity, and stress tolerance. *Abies ziyuanensis* L. K. Fu & S. L. Mo (Pinaceae) is an endangered conifer endemic to China, and its persistence may depend on its interactions with its belowground microbiome. However, how soil-borne bacterial functional groups respond to, and potentially support, *A. ziyuanensis* remains unclear. Based on amplicon high-throughput sequencing data of the 16S rRNA gene and soil physicochemical properties, the bacterial community structure in the rhizosphere soil of *A. ziyuanensis* in Yinzhushan National Nature Reserve in Guangxi Zhuang Autonomous Region, China, was analyzed, and the potential ecological functions and phenotypic characteristics of the bacterial community were predicted to determine the functional taxa characteristics (nitrogen cycle, phototrophy, and chemoheterotrophy) and dominant soil environmental factors. Proteobacteria, Acidobacteria, Actinobacteria, Planctomycetes, Verrucomicrobia, and Chloroflexi were the dominant bacterial taxa in the *A. ziyuanensis* rhizosphere soil, and all bacteria were significantly positively correlated with soil NO_3^- -N ($R = 0.47$, $p = 0.0079$). Based on FAPROTAX, the *A. ziyuanensis* rhizosphere soil bacterial community had chemoheterotrophic-related functions, which were more prevalent than nitrogen cycle- and phototrophic-related functions, and the relative abundance of bacteria with nitrogen cycle-related functions was higher than that of those with phototrophic functions. The nitrogen nutrient- and phototrophic-related functional taxa in the rhizosphere soil bacterial community had significant correlations with soil physicochemical properties, whereas the chemoheterotrophic-related functional taxa did not show a significant correlation. Based on BugBase phenotype prediction, Acidobacteria, Proteobacteria, and Chloroflexi made the greatest contribution to the phenotype, with pathogenic and stress tolerance being the most important phenotypes. The pathogenic and stress-tolerant bacteria all belonged to Proteobacteria. The rhizosphere bacteria exhibited rich diversity and dominated several biogeochemical cycling processes. This study identifies beneficial rhizosphere bacteria of *A. ziyuanensis*, providing a theoretical basis for conserving soil bacterial diversity and guiding the targeted recruitment of functional bacteria by the endangered plant.



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1. Introduction

Soil microbial communities are the most diverse repositories of underground genes and metabolites on Earth [1]. The rhizosphere refers to the 1–2 mm soil region influenced by living roots, in which microbial density is higher than in ordinary soil and which regulates the interactions between plants and microorganisms [2,3]. The root microbiome is among the most abundant microbial ecosystems on the planet. Within this ecosystem, bacteria comprising the microbiome play pivotal roles in regulating plant growth and development [4–7] and have various ecological functions across different niches, including biogeochemical cycles involving carbon (C), nitrogen (N), and sulfur and organic matter breakdown [8–11]. Specifically, phototrophic and chemoheterotrophic bacteria use both organic and inorganic C sources to support their growth and development [12]. Consequently, bacteria decompose recalcitrant organic matter and drive the C cycle while also participating in the N cycle, with some even fixing atmospheric dinitrogen to supply biologically available N for other organisms [10,13,14]. Although the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database does not determine the functional phenotypes of all taxa in a community, previous studies have shown that it is useful for predicting functions related to biogeochemical dynamics in environmental samples [15,16], especially N and C cycles [17,18]. For example, FAPROTAcX has been used to determine the effect of microbial inoculation and fertilization on the functional soil bacteria involved in the C and N cycles, revealing the significant effects of straw return and N fertilizer on hydrocarbon degradation and N fixation [19]. Building on these insights, understanding the structure and functional potential of rhizosphere bacterial communities is crucial in endangered plants, as their survival often depends on the stability and functionality of their associated bacterial communities.

Root exudates (e.g., sugars, amino acids, phenols) act as chemical attractants, recruiting specific taxa while inhibiting others, thereby shaping the root-associated microbiome to match the plant's immediate nutritional and defensive needs [3]. Moreover, the composition of rhizosphere bacterial communities is an indicator of the plant health status [20]. Most plant rhizosphere bacterial communities have bacteria in common with environmental soil bacterial communities, and bacteria can interact with and be transferred between plants and the environment, significantly affecting plant health by participating in biological processes, such as nutrient absorption, metabolism, energy flow, material cycling, and growth and development [21]. Therefore, the status of bacterial communities in the plant rhizosphere serves as an early indicator of plant health. Bacterial phenotypes have been used to determine the health status of bacterial communities in the plant rhizosphere [22]. BugBase phenotype prediction precisely meets this requirement [23]. The study of bacterial community variations along the natural salinity gradient in the Kelar Yergul River Basin, northwestern Taklamakan Desert, China, has shown that the phenotypes of aerobic, facultative anaerobic, Gram-positive, and stress-tolerant bacteria in the surface (0–30 cm) and deep soil (30–60 cm) showed a significant negative correlation with salinity [24]. Based on the BugBase phenotypes (oxygen utilization, mobile elements, potential pathogenicity, and oxidative stress tolerance), bacteria can be identified as potential threats to plant health. Predicting the regulation of rhizosphere bacterial communities based on these phenotypes can enable plant disease control and biodiversity conservation.

In forest ecosystems, pine plants rely on their microbial communities to acquire nutrients, enhance drought resistance, and defend against pathogen invasion [4]. *Abies ziyuanensis* L. K. Fu & S. L. Mo is an endangered plant endemic to Yin Zhu Laoshan in Shenbaotang (SBT) and Sanjiaohutang (SJHT), Guangxi, China. It belongs to the Pinaceae family and was first discovered in 1977 [25]. Unfortunately, over the past few decades, the wild *A. ziyuanensis* population has continuously declined, and their distribution area has

progressively shrunk due to global climate change, human activities, farmland expansion, forestland degradation, habitat fragmentation, and environmental deterioration [26]. In recent years, the planting of *A. ziyuanensis* has increased. However, the main issues with afforestation are the seedling survival rate and their pest and disease resistance. Unsuitable soil conditions may cause functional loss and phenotypic decline of the species, reducing the survival rate of *A. ziyuanensis* seedlings, which may explain its endangerment [27]. Therefore, a comprehensive study of the rhizosphere bacteria can provide an understanding of the conditions required for the survival of the endangered plant *A. ziyuanensis*, thus aiding its conservation, as the results can be used to evaluate conservation effectiveness and guide conservation practices. We hypothesize that *A. ziyuanensis* selectively enriches a distinct suite of rhizosphere bacteria, giving rise to a functionally specialized community whose composition and activity are strongly governed by soil physicochemical conditions. Accordingly, this study focused on two *A. ziyuanensis* populations from SBT and SJHT in Yinzhu Laoshan, China, and used high-throughput sequencing data obtained from soil samples of each forest stand to (i) determine the taxonomic composition of their microbiota, (ii) predict the ecological functions and phenotypes of these microorganisms, and (iii) relate bacterial community patterns to soil physicochemical properties. Ultimately, the results of this study provide a basis for selecting appropriate breeding sites for *A. ziyuanensis* seedlings and restoring the habitats of wild populations and a scientific reference for evaluating the potential soil ecological functions of this region.

2. Materials and Methods

2.1. Overview of the Research Area and Soil Sample Collection

The study site is located within the Yinzhu Laoshan National Nature Reserve in Guilin, Guangxi Zhuang Autonomous Region, China (110°32'42"–110°35'06" E, 26°15'05"–26°19'15" N), which is within the mid-subtropical monsoon climate zone. The region has an average annual temperature of 13.1 °C, an extreme low temperature of −11.9 °C, and an extreme high temperature of 34 °C. The region experiences a total annual precipitation of 2065 mm, with the rainy season occurring from March to August, accounting for 71% of the total annual rainfall. The dry season lasts from September to February of the following year, and the average annual relative humidity reaches 85%. The topography is characterized by mid-mountain ranges, and the zonal soil is primarily yellow-brown earth developed from granite. *Abies ziyuanensis* L. K. Fu & S. L. Mo is distributed at elevations ranging from 1600 to 1850 m. Located along the cold current in the Xiang-Gui Corridor, the microclimate is characterized by frequent fog and clouds, high humidity, prolonged low-temperature periods, and frequent frost and snow during winter [26]. The Shenbaotang (SBT) population (110°33'33"–110°33'39" E, 26°15'05"–26°15'12" N) of this species occurs at 1754–1822 m a.s.l.; its trees have a DBH of 5–37 cm and a height of 10–23 m, and the dominant accompanying plants are *Fargesia spathacea* Franch, *Eurya semiserrulata* Hung T. Chang, and *Quercus glauca* Thunb. Meanwhile, the Sanjiaohutang (SJHT) population (110°33'18"–110°33'34" E, 26°15'21"–26°15'48" N) ranges from 1731 to 1940 m a.s.l., and its trees have a DBH of 3–26 cm and a height of 0.8–20 m. The dominant accompanying plants are *Fargesia spathacea* Franch, *Rhododendron latoucheae* Franch, and *Schima superba* Gardner & Champ.

This study was conceived in late 2022, and sampling was completed in July 2023, after which samples were immediately transported to the laboratory for physicochemical analyses and high-throughput sequencing. Data processing and manuscript preparation have continued to the present. We selected two natural *A. ziyuanensis* populations, situated in SBT and SJHT within the nature reserve, as our study subjects. At each of the sample sites, 15 healthy *A. ziyuanensis* trees were randomly selected from relatively uniform slopes.

The litter and weeds around the target trees were removed, and approximately 500 g of soil was collected at a depth of 0–20 cm using a soil auger (inner diameter 5.0 cm) and a shovel. The soil was placed into sterile zip-lock bags, and three soil samples were collected at 1 m intervals in a triangle pattern around each tree and mixed into one composite sample. Sampling information was recorded, and the samples were transported to the laboratory using portable insulated boxes with bio-ice packs. The fresh mixed soil samples were divided into three portions in the laboratory: one portion was sieved through a 2 mm steel mesh and stored at -80°C for high-throughput sequencing of soil bacteria; another portion was sieved through a 2 mm steel mesh and stored at 4°C for the determination of ammonium nitrogen ($\text{NH}_4^{+}\text{-N}$), nitrate nitrogen ($\text{NO}_3^{-}\text{-N}$), and microbial biomass C (MBC) and N (MBN) contents; and the third portion was air-dried naturally indoors and sieved through a 0.25 mm mesh to analyze the soil physicochemical properties.

2.2. Determination of Soil Physicochemical Properties

The soil water content (SWC) was determined using the gravimetric method (YH-C30002 electronic balance, Sartorius Scientific Instruments, Beijing, China), and the pH was measured using the potentiometric method (water-to-soil ratio of 2.5:1; ST2100 pH meter, OHAUS Instruments, Shanghai, China). The soil organic carbon (SOC) content was determined using the potassium dichromate oxidation-external heating method. The total nitrogen (TN) content was determined using sulfuric acid digestion and the Kjeldahl method. The $\text{NH}_4^{+}\text{-N}$ and $\text{NO}_3^{-}\text{-N}$ contents were determined using the indophenol blue and phenol disulfonic acid colorimetric methods, respectively (TU-1901 UV visible spectrophotometer, Beijing Purkinje General Instrument Co., Ltd., Beijing, China). The soil available nitrogen (AN) content was measured using the alkali hydrolysis diffusion method, and the soil total phosphorus (TP) content was determined using the sodium hydroxide fusion-molybdenum antimony colorimetric method (TU-1901 UV visible spectrophotometer, Beijing Purkinje General Instrument Co., Ltd.). The available phosphorus (AP) content was measured using the sodium bicarbonate/sodium fluoride-hydrochloric acid extraction-molybdenum antimony colorimetric method (TU-1901 UV visible spectrophotometer, Beijing Purkinje General Instrument Co., Ltd.). The soil total potassium (TK) content was determined using the NaOH fusion-flame photometry method (TAS-990F flame atomic absorption spectrophotometer, Beijing Purkinje General Instrument Co., Ltd.), and the soil available potassium (AK) content was measured using the ammonium acetate extraction-flame photometry method (TAS-990F flame atomic absorption spectrophotometer, Beijing Purkinje General Instrument Co., Ltd.). The soil MBC and MBN were determined using the chloroform fumigation extraction method and analyzed with a carbon-nitrogen analyzer (Sartorius Scientific Instruments) following the method of Bao [28].

2.3. Soil Bacterial DNA Extraction, PCR Amplification, and High-Throughput Sequencing

DNA extraction was performed on 30 samples using an E.Z.N.ATM Mag-Bind Soil DNA Kit (M5635-02, OMEGA, Cambridge, MA, USA). The genomic DNA was precisely quantified using a Qubit[®] 4.0 DNA Assay Kit (Q33238, Thermo Fisher, Waltham, MA, USA) to determine the amount of DNA to be added to the PCR. PCR amplification (ETC 811, Beijing Dongsheng Innovation Biotechnology Co., Beijing, China) was performed to amplify the V3–V4 region of the bacterial 16S rRNA gene with two rounds of amplification, using the primers Nobar_341F (CCTACGGGNGGCWGCAG) and Nobar_805R (GACTACHVGGGTATCTAATCC). The reaction system for the two rounds of PCR amplification contained the following: 15 μL of $2 \times$ Hieff[®] Robust PCR Master Mix (10105ES03, Yeasen, Gaithersburg, MD, USA), 1 μL of Bar-PCR primer F, 1 μL of Primer R, 10–20 ng

of PCR products, and 9–12 μL of H_2O , with a total volume of 30 μL . The conditions for the first round of PCR were as follows: 94 °C for 3 min $\rightarrow$ (94 °C for 30 s $\rightarrow$ 45 °C for 20 s $\rightarrow$ 65 °C for 30 s) $\times$ 5 cycles $\rightarrow$ (94 °C for 20 s $\rightarrow$ 55 °C for 20 s $\rightarrow$ 72 °C for 30 s) $\times$ 20 cycles $\rightarrow$ 72 °C for 5 min $\rightarrow$ hold at 10 °C. The conditions for the second round of PCR were as follows: 95 °C for 3 min $\rightarrow$ (94 °C for 20 s $\rightarrow$ 55 °C for 20 s $\rightarrow$ 72 °C for 30 s) $\times$ 5 cycles $\rightarrow$ 72 °C for 5 min $\rightarrow$ hold at 10 °C. The library size was determined using 2% agarose gel electrophoresis, and the library concentration was measured using a Qubit 4.0 Fluorometer (Q33238, Thermo Fisher, Waltham, MA, USA), all samples were mixed in equal amounts in a 1:1 ratio. Library construction and high-throughput sequencing were performed by Sangon Biotechnology Co., Ltd. (Shanghai, China) using the Illumina Miseq PE300 platform.

2.4. Bioinformatics Analysis

The raw image data files obtained from Illumina MiseqTM/HiseqTM (Sangon Biotechnology Co., Ltd., Shanghai, China) were converted to raw sequencing reads via base calling analysis. Cutadapt 1.18 [29] was used to remove the primer and adapter sequences, and PEAR 0.9.8 [30] was used to merge paired-end reads into a single sequence based on their overlap. The sequences were demultiplexed according to barcode tags to distinguish samples. PRINSEQ 0.20.4 [31] was then used to perform quality control and filter the data of each sample, yielding high-quality effective data. The non-redundant sequences (excluding singletons) were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using Usearch 11.0.667 software [32,33], and chimeras were removed to identify representative OTUs. The representative OTU sequences were annotated for species classification using mothur 1.43.0 (classify.seqs) [34] by comparing them against the Silva 138.2 classifier database (<https://www.arb-silva.de/> (accessed on 21 April 2024)) [35]. The OTU table and species annotation information were merged, and the functional groups of bacterial communities (Table 1) were analyzed in each sample through comparison with the FAPROTAX 1.2.1 database [15]. Additionally, the phenotypic characteristics of bacterial communities were determined in each sample using the BugBase 0.1.0 database [36].

Table 1. Bacterial functional groups of all soil samples [37].

Types	Functions
Nitrogen cycle	Aerobic ammonia oxidation
	Aerobic nitrite oxidation
	Nitrification
	Nitrate denitrification
	Nitrite denitrification
	Nitrous oxide denitrification
	Nitrogen fixation
	Nitrate ammonification
	Nitrite ammonification
	Nitrite respiration
	Nitrate respiration
	Nitrate reduction
	Nitrogen respiration
Phototrophy	Anoxygenic sulfur-oxidizing photoautotrophy
	Anoxygenic photoautotrophy
	Photoautotrophy
	Photoheterotrophy
	Phototrophy
Chemoheterotrophy	Chemoheterotrophy
	Aerobic chemoheterotrophy

2.5. Statistical Analysis

A *t*-test was used to determine the significance of differences in soil physicochemical properties and alpha diversity indices between the two sites. Beta diversity was visualized using nonmetric multidimensional scaling (NMDS) based on the unweighted Unifrac distance. Random Forest analysis was performed to determine the importance of species at the phylum level between the two sites. STAMP 2.1.3 software [38] was used to identify differences in species at the phylum level between the two sites. Linear regression analysis was performed to identify correlations between bacterial communities and environmental factors. The wilcox.test was used to evaluate the significance of differences in soil bacterial functional groups between the two sites. The data met the conditions of normal distribution, and Pearson correlation analysis was conducted to determine the influence of soil physicochemical properties on bacterial functional groups in *A. ziyuanensis* soil. Relevant calculations were performed using Excel 2016, and figures were generated using GraphPad Prism 8.3 software.

3. Results

3.1. Physicochemical Properties of *A. ziyuanensis* Rhizosphere Soil

The *t*-test revealed (Table 2) that SWC, pH, TN, AN, NO_3^- -N, NH_4^+ -N, SOC, TP, AP, MBC, and MBN did not significantly differ ($p > 0.05$) between the distribution sites of the two *A. ziyuanensis* populations. However, TK and AK were significantly higher in SBT than in SJHT ($p < 0.05$). This indicates that the soil fertility of these two populations is comparable, but the differences between TK and AK may serve as drivers for rhizosphere bacterial communities.

Table 2. Soil physicochemical properties (mean $\pm$ standard deviation, $n = 15$).

Physicochemical Properties	SBT	SJHT	Significance
SWC (%)	40.54 $\pm$ 9.85	48.83 $\pm$ 16.47	-
pH	4.30 $\pm$ 0.22	4.38 $\pm$ 0.19	-
TN (g/kg)	5.57 $\pm$ 1.14	5.38 $\pm$ 1.98	-
AN (mg/kg)	285.60 $\pm$ 68.97	279.88 $\pm$ 118.87	-
NO_3^- -N (mg/kg)	5.01 $\pm$ 6.43	8.74 $\pm$ 11.71	-
NH_4^+ -N (mg/kg)	31.07 $\pm$ 13.60	30.90 $\pm$ 13.05	-
SOC (g/kg)	72.71 $\pm$ 18.54	64.15 $\pm$ 15.88	-
TP (g/kg)	0.44 $\pm$ 0.30	0.71 $\pm$ 0.59	-
AP (mg/kg)	3.44 $\pm$ 2.06	4.66 $\pm$ 6.36	-
TK (g/kg)	22.27 $\pm$ 4.28	17.55 $\pm$ 4.08	**
AK (mg/kg)	95.73 $\pm$ 16.44	80.80 $\pm$ 20.60	*
MBC (mg/kg)	1062.40 $\pm$ 382.04	1092.67 $\pm$ 501.95	-
MBN (mg/kg)	456.00 $\pm$ 179.84	365.47 $\pm$ 117.39	-

Note: Shenbaotang (SBT) and Sanjiaohutang (SJHT) refer to the sites. AK, available potassium; AN, available nitrogen; AP, available phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; NH_4^+ -N, ammonium nitrogen; NO_3^- -N, nitrate nitrogen; SOC, soil organic carbon; SWC, soil water content; TK, total potassium; TN, total nitrogen; TP, total phosphorus. *: $p < 0.05$; **: $p < 0.01$.

3.2. Soil Bacterial Alpha Diversity in the Rhizosphere Soil of *A. ziyuanensis*

Based on QC and the 97% sequence similarity level, it can be known that the library coverage of rhizosphere bacteria in both populations exceeds 99%. This indicates that the samples can cover most of the bacteria in the soil and truly reflect the information of the soil bacterial community (Supplementary Table S1 and Supplementary Figure S1).

There were no significant differences in the OTUs, Coverage, Abundance-based Coverage Estimator (ACE), Shannon, Chao, or Simpson indices between SBT and SJHT according to a *t*-test ($p > 0.05$, $n = 15$) (Figure 1), indicating that there were no differences in the species diversity of the rhizosphere soil of *A. ziyuanensis* from Yinzhua Laoshan.

3.3. Soil Bacterial Beta Diversity in the Rhizosphere Soil of *A. ziyuanensis*

NMDS analysis showed that the bacterial community structure was similar between SBT and SJHT (Figure 2A). The unweighted Unifrac diversity distance classified the 30 samples into different groups, and the bacterial community in the rhizosphere soils of *A. ziyuanensis* from Yinzhong Laoshan was not clustered according to the different sampling sites (Figure 2B). The overall composition of rhizosphere bacterial communities of *A. ziyuanensis* tends to homogenize at both sites, but there are subtle differentiations at the phylogenetic level.

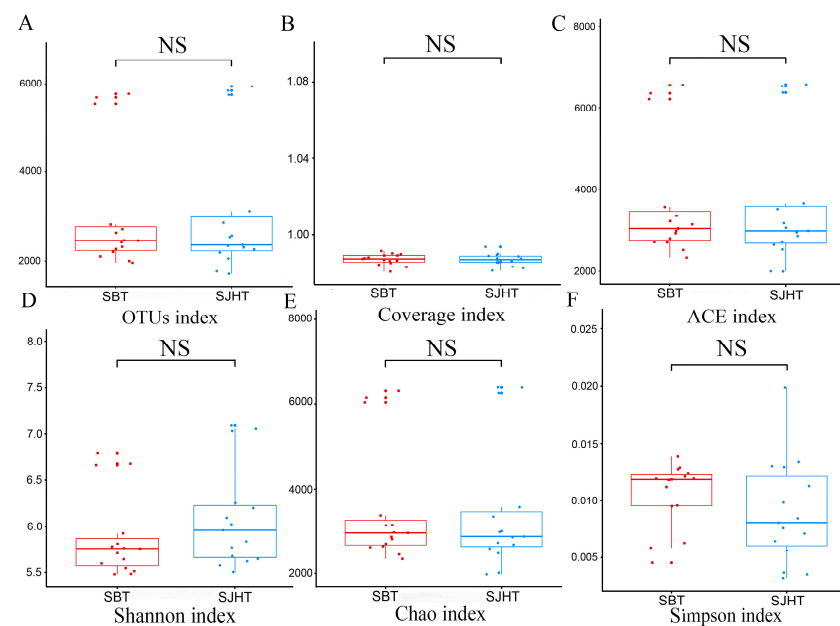


Figure 1. Soil bacterial alpha diversity in the *Abies ziyuanensis* rhizosphere. Note: Shenbaotang (SBT) and Sanjiaohutang (SJHT) refer to the sampling sites. NS, $p > 0.05$; (A) OTUs index; (B) Coverage index; (C) ACE index; (D) Shannon index; (E) Chao index; (F) Simpson index.

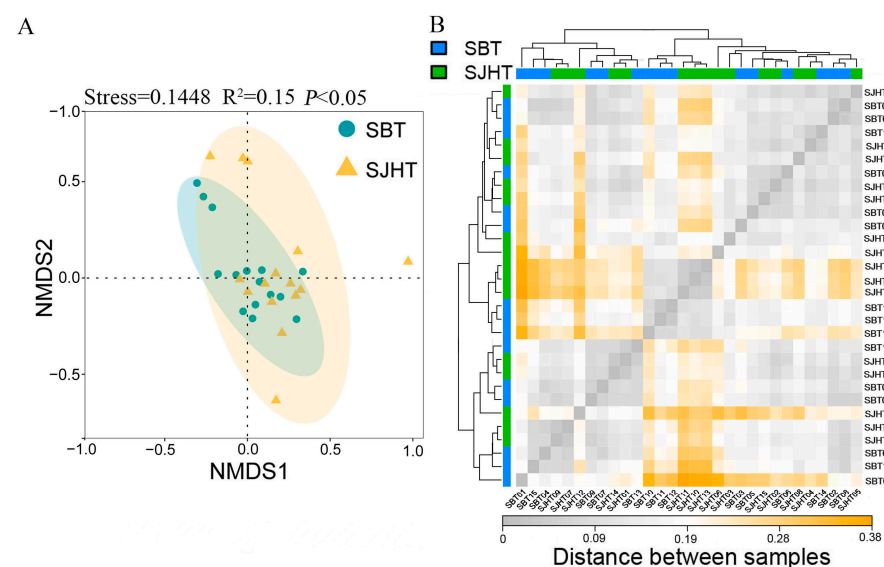


Figure 2. (A) Non-metric multidimensional scaling analysis (NMDS) based on the upper unweighted Unifrac distance; (B) clustering heatmap based on the unweighted Unifrac distance. Note: SBT01 to SBT15 and SJHT01 to SJHT15 represent rhizosphere samples from 15 plants analyzed at the two sites, Shenbaotang (SBT) and Sanjiaohutang (SJHT).

3.4. Characterization of the Bacterial Composition of the Rhizosphere Soil of *A. ziyuanensis* from Yinzhong Laoshan

From 30 samples, 18,627 bacterial OTUs were obtained, belonging to 38 phyla, 106 classes, 187 orders, 382 families, and 903 genera. Sequence annotation of the samples revealed that the dominant soil bacterial phyla in the rhizosphere soil of *A. ziyuanensis* were Proteobacteria (average relative abundance: SBT 35.65%, SJHT 36.64%), Acidobacteria (SBT 34.90%, SJHT 30.44%), Actinobacteria (SBT 7.73%, SJHT 6.24%), Actinomyces (SJHT 34.90%, SJHT 30.44%), Actinobacteria (SBT 7.73%, SJHT 6.24%), Planctomycetes (SBT 4.87%, SJHT 5.14%), Verrucomicrobia (SBT 4.40%, SJHT 4.45%), and Chloroflexi (SBT 2.03%, SJHT 2.92%). The dominant phyla accounted for 89.58 and 89.69% of the total species relative abundance at SBT and SJHT, respectively. (Figure 3A,B).

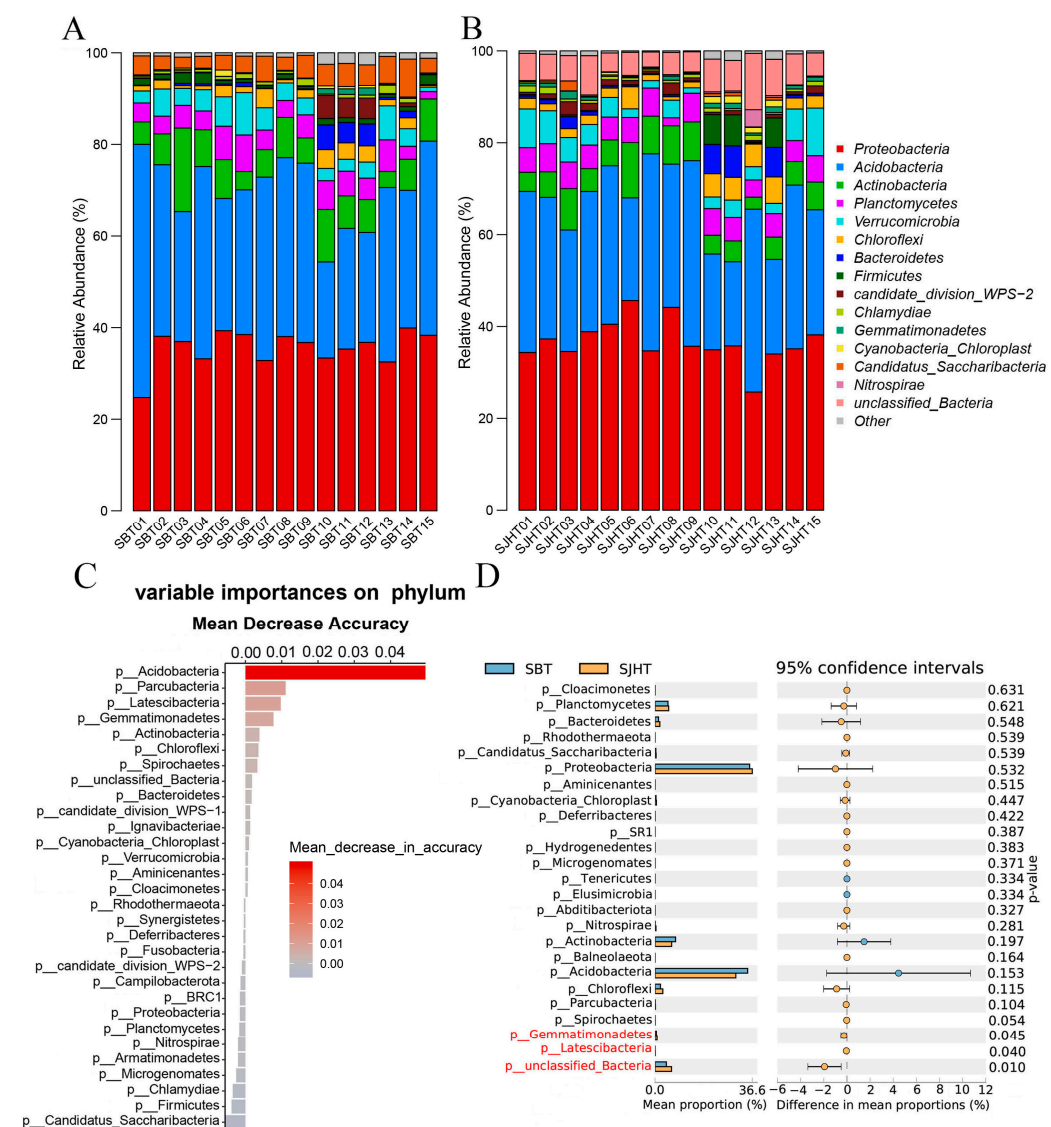


Figure 3. (A,B) Species composition of rhizosphere soil bacterial communities at the phylum level; (C) random Forest analysis; and (D) STAMP analysis of variance. Note: SBT01 to SBT15 and SJHT01 to SJHT15 represent rhizosphere samples from 15 plants analyzed at the two sites, Shenbaotang (SBT) and Sanjiaohutang (SJHT). Red font indicates $p < 0.05$, and black font indicates $p > 0.05$.

Random Forest analysis revealed that Acidobacteria had the highest significance, followed by Parcubacteria, Latescibacteria, and Gemmatimonadetes (Figure 3C). STAMP difference analysis revealed that only three phyla were significantly different between SBT

and SJHT, namely Gemmatimonadetes, Latescibacteria, and unclassified bacteria. Their relative abundance was higher in SJHT than in SBT ($p < 0.05$) (Figure 3D).

3.5. Sequential Regression Analysis of *A. ziyuanensis* Rhizosphere Soil Bacteria and Environmental Factors

Principal coordinate analysis (PCoA) was used to analyze the correlation patterns between bacterial phyla and environmental factors based on the PCo1 axis. Bacterial phyla showed significant positive correlations with soil NO_3^- -N ($R = 0.47$, $p = 0.0079$) (Figure 4). Positive but non-significant correlations were observed with TN ($R = 0.051$, $p = 0.78$), TP ($R = 0.1$, $p = 0.58$), AP ($R = 0.028$, $p = 0.88$), NH_4^+ -N ($R = 0.064$, $p = 0.73$), pH ($R = 0.21$, $p = 0.25$), and MBC ($R = 0.076$, $p = 0.68$). Negative but non-significant correlations were found with TK ($R = -0.22$, $p = 0.22$), AN ($R = -0.083$, $p = 0.65$), AK ($R = -0.084$, $p = 0.65$), SOC ($R = -0.12$, $p = 0.52$), SWC ($R = -0.11$, $p = 0.56$), and MBN ($R = -0.34$, $p = 0.06$) (Figure 4).

3.6. Prediction of the Functions of *A. ziyuanensis* Rhizosphere Soil Bacteria

FAPROTAX was used to predict the ecological functions of the top 22 bacterial species comprising the total abundance in the *A. ziyuanensis* rhizosphere soil from Yinzhu Laoshan, and a function histogram was generated (Figure 5A). The prediction results showed that the following functions were relatively frequent in the *A. ziyuanensis* rhizosphere soil bacteria from Yinzhu Laoshan: chemoheterotrophy, aerobic_chemoheterotrophy, N fixation, intracellular_parasites, and fermentation.

The 18 main functions were categorized into 3 groups: functions related to phototrophy, N cycling, and chemical heterotrophy. Functions related to chemical heterotrophy occurred more frequently in the rhizosphere soil of *A. ziyuanensis* than those related to N cycling and phototrophy. The relative abundance of bacteria with functions related to N cycling was also higher than that of bacteria with functions related to phototrophic functions. These three function categories were not significantly different ($p < 0.05$) between SBT and SJHT (Figure 5B).

3.7. Correlation Between the Functional Groups of *A. ziyuanensis* Rhizosphere Soil Bacteria and Soil Environmental Factors

Pearson correlation analysis showed that nitrogen_fixation related to the nitrogen cycle in SBT was significantly negatively correlated with AK ($p < 0.05$). Phototrophy related to anoxygenic_photoautotrophy, anoxygenic_photoautotrophy_S_oxidizing, and photoautotrophy were significantly positively correlated with AP ($p < 0.05$) but highly significantly negatively correlated with SWC ($p < 0.01$). However, the chemoheterotrophy was not significantly correlated with the soil physicochemical properties ($p > 0.05$) (Figure 6A).

In SJHT, nitrogen_fixation related to N nutrition was significantly negatively correlated with AK, AN, and TN ($p < 0.05$) but significantly positively correlated with MBN ($p < 0.05$). Aerobic_nitrite_oxidation and nitrification were significantly positively correlated with TN ($p < 0.05$), highly significantly positively correlated with AN ($p < 0.01$), and extremely significantly positively correlated with AP ($p < 0.001$). Nitrate_reduction and nitrite_respiration were significantly positively correlated with NO_3^- -N ($p < 0.05$), whereas photonutrition-related photoheterotrophy and phototrophy were significantly negatively correlated with NO_3^- -N ($p < 0.05$). Chemical nutrition-related functions were not significantly correlated with the soil physicochemical properties ($p > 0.05$) (Figure 6B).

In general, the functional groups related to N and phototrophic nutrition in the rhizosphere soil bacteria of *A. ziyuanensis* from Yinzhu Laoshan had more or less significant correlations with the soil physicochemical properties. However, the functional groups

related to chemical nutrition did not show significant correlations with the soil physico-chemical properties.

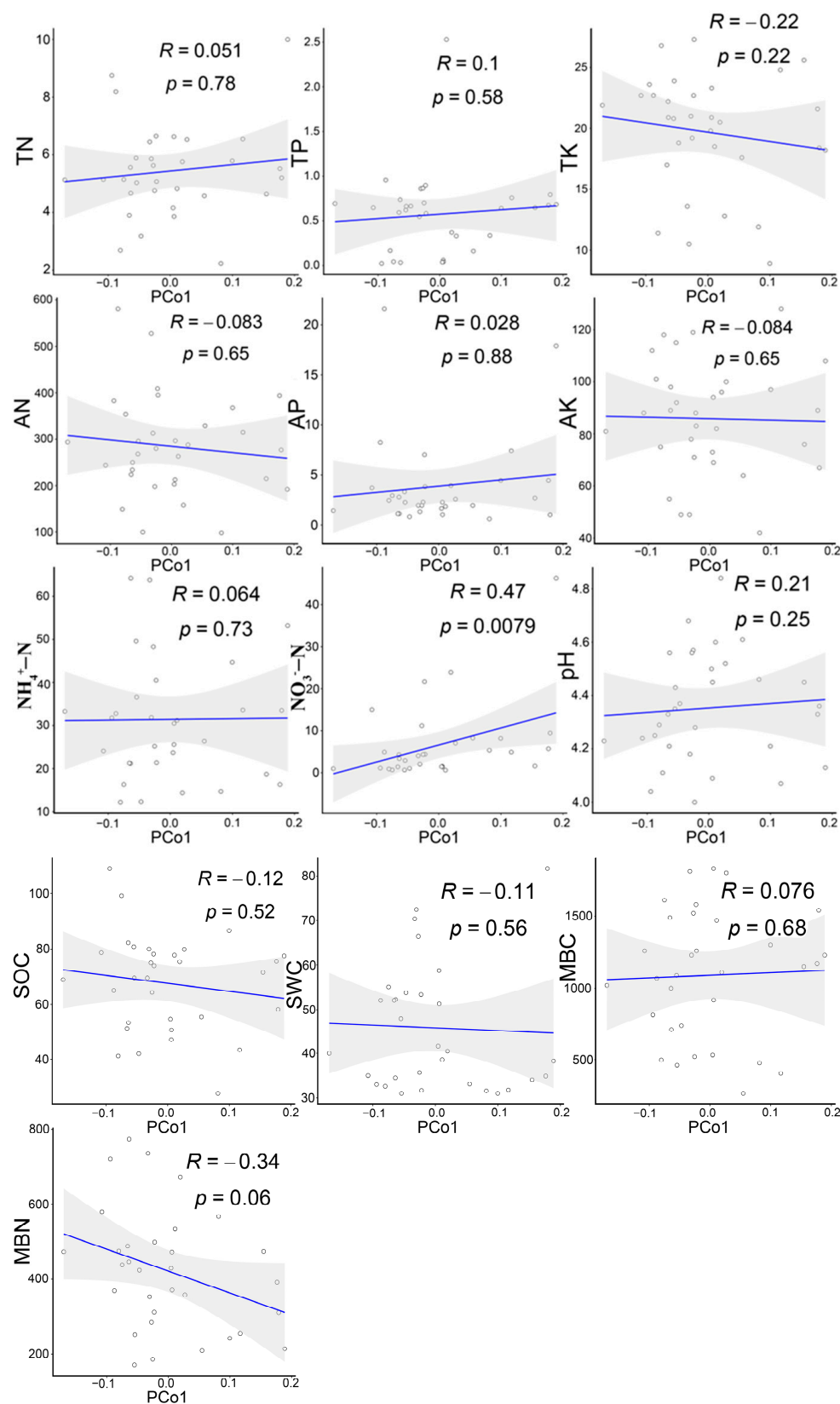


Figure 4. Sequential regression analysis of environmental factors in the rhizosphere soil bacterial community of *Abies ziyuanensis* (based on the phylum level).

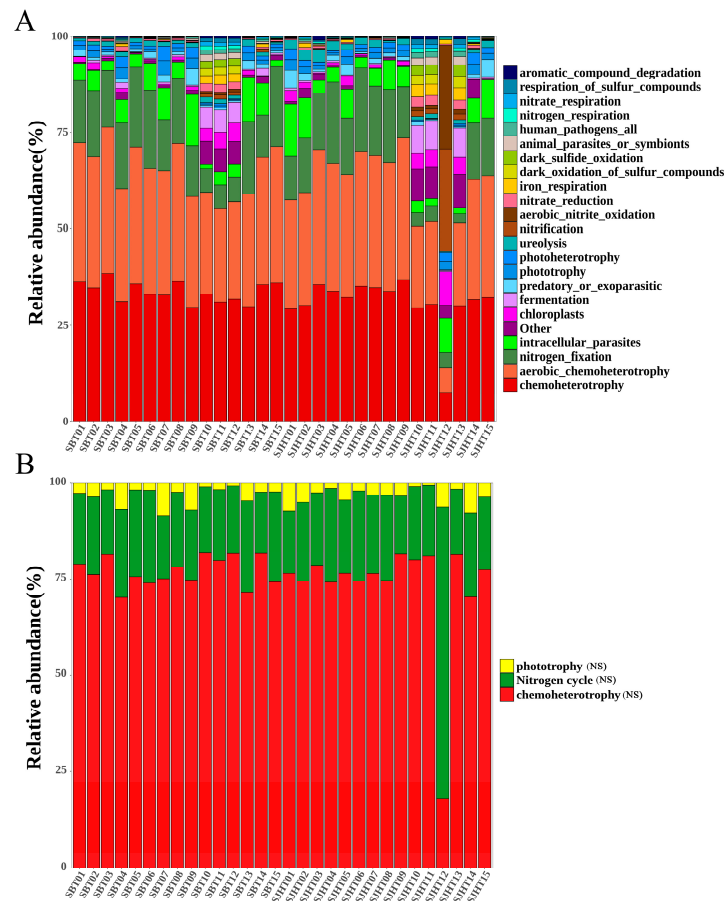


Figure 5. (A) Relative abundance of functional soil bacterial taxa in the rhizosphere of *Abies ziyuanensis*; (B) relative abundance of major functional groups. Note: SBT01 to SBT15 and SJHT01 to SJHT15 represent rhizosphere samples from 15 plants analyzed at the two sites, Shenbaotang (SBT) and Sanjiaohutang (SJHT). NS, no significant difference between SBT and SJHT.

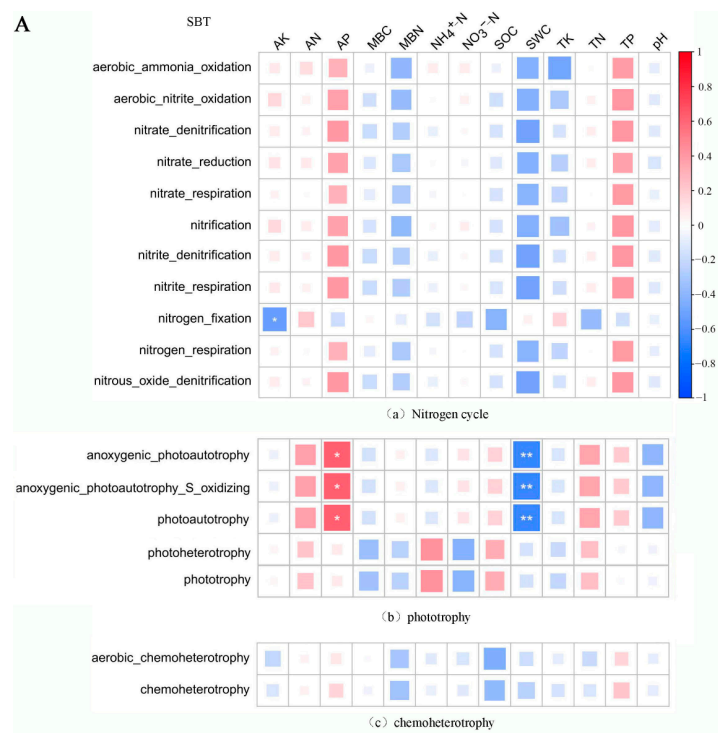


Figure 6. Cont.

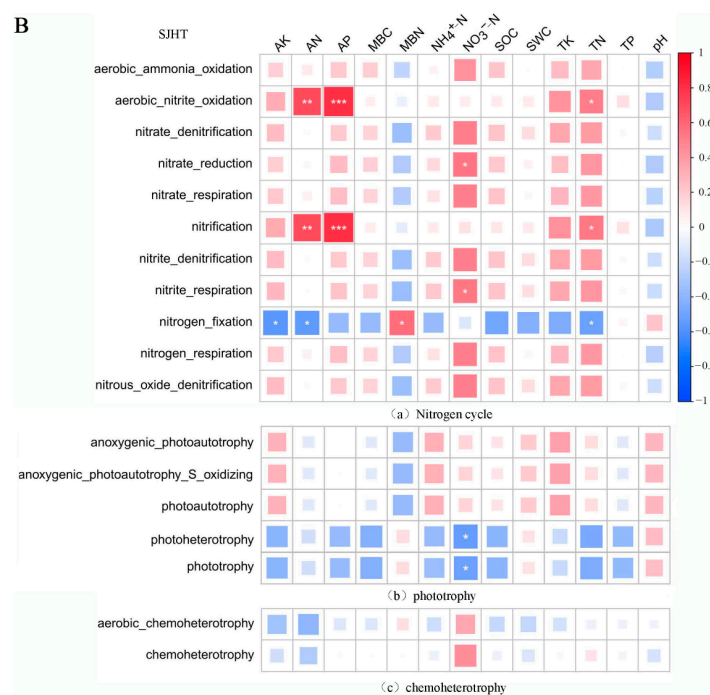


Figure 6. Pearson correlation analysis between gene abundance of soil bacterial functional groups and soil physicochemical factors. Note: Shenbaotang ((A) SBT) and Sanjiaohutang ((B) SJHT) refer to the sampling sites. Red and blue indicate positive and negative correlations, respectively, between two variables, and the darker the color, the stronger the relationship; *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

3.8. Phenotype Prediction of *A. ziyuanensis* Rhizosphere Soil Bacteria

Phenotypic predictions were conducted for the rhizosphere soil bacteria of *A. ziyuanensis* from Yinzhong Laoshan using the BugBase analysis tool. The predicted phenotypic categories included Gram-positive, Gram-negative, biofilm-forming, pathogenic, mobile element-containing, oxygen-utilizing (including aerobic, anaerobic, and facultatively anaerobic), and stress-tolerant bacteria, totaling seven categories (Figure 7). There were no significant differences in these seven phenotypic categories between SBT and SJHT ($p > 0.05$).

As shown in Figure 8, at the phylum level, the top 10 species in terms of total relative abundance were analyzed for their phenotypic contributions. For the Gram-positive phenotype, Actinobacteria and Chloroflexi contributed 0.04530642 and 0.016649794, respectively, in SBT, and Actinobacteria, Chloroflexi, and Firmicutes contributed 0.039247835, 0.024385853, and 0.005940331, respectively, in SJHT. For the Gram-negative phenotype, Acidobacteria and Proteobacteria contributed 0.568929301 and 0.209813466, respectively, in SBT, while they contributed 0.506131188 and 0.23024516, respectively, in SJHT. Proteobacteria contributed 0.000689869 and 0.000852806 to the biofilm-forming phenotype in SBT and SJHT, respectively. For the anaerobic phenotype, Acidobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Planctomycetes, Proteobacteria, and Verrucomicrobia contributed 0.005625425, 0.001426563, 0.001370368, 0.00315881, 0.006429683, 0.003681964, and 0.001425235, respectively, in SBT, and Acidobacteria, Armatimonadetes, Bacteroidetes, Chloroflexi, Firmicutes, Planctomycetes, and Proteobacteria contributed 0.0095097, 0.001230014, 0.002019869, 0.002187087, 0.004168173, 0.008365123, and 0.005545192, respectively, in SJHT. Acidobacteria contributed 0.563303877 to the aerobic phenotype in SBT, and Acidobacteria and Proteobacteria contributed 0.496621488 and 0.066276119, respectively, to this phenotype in SJHT. Proteobacteria contributed 0.088033265 and 0.084976297 to the facultative anaerobic phenotype, 0.000709669 and 0.000889785 to the pathogenic phenotype, 0.00069388

and 0.000860632 to the stress-tolerant phenotype, and 0.000121758 and 0.000404433 to the mobile element-containing phenotype in SBT and SJHT, respectively.

Overall, the bacterial communities in the rhizosphere soil of the old-growth *Abies* resources in Yinzhu Laoshan were predominantly composed of several phyla, namely Acidobacteria, Proteobacteria, and Chloroflexi, that contributed significantly to the phenotypes. Among these, the pathogenic and stress-tolerant groups both belonged to the phylum Proteobacteria.

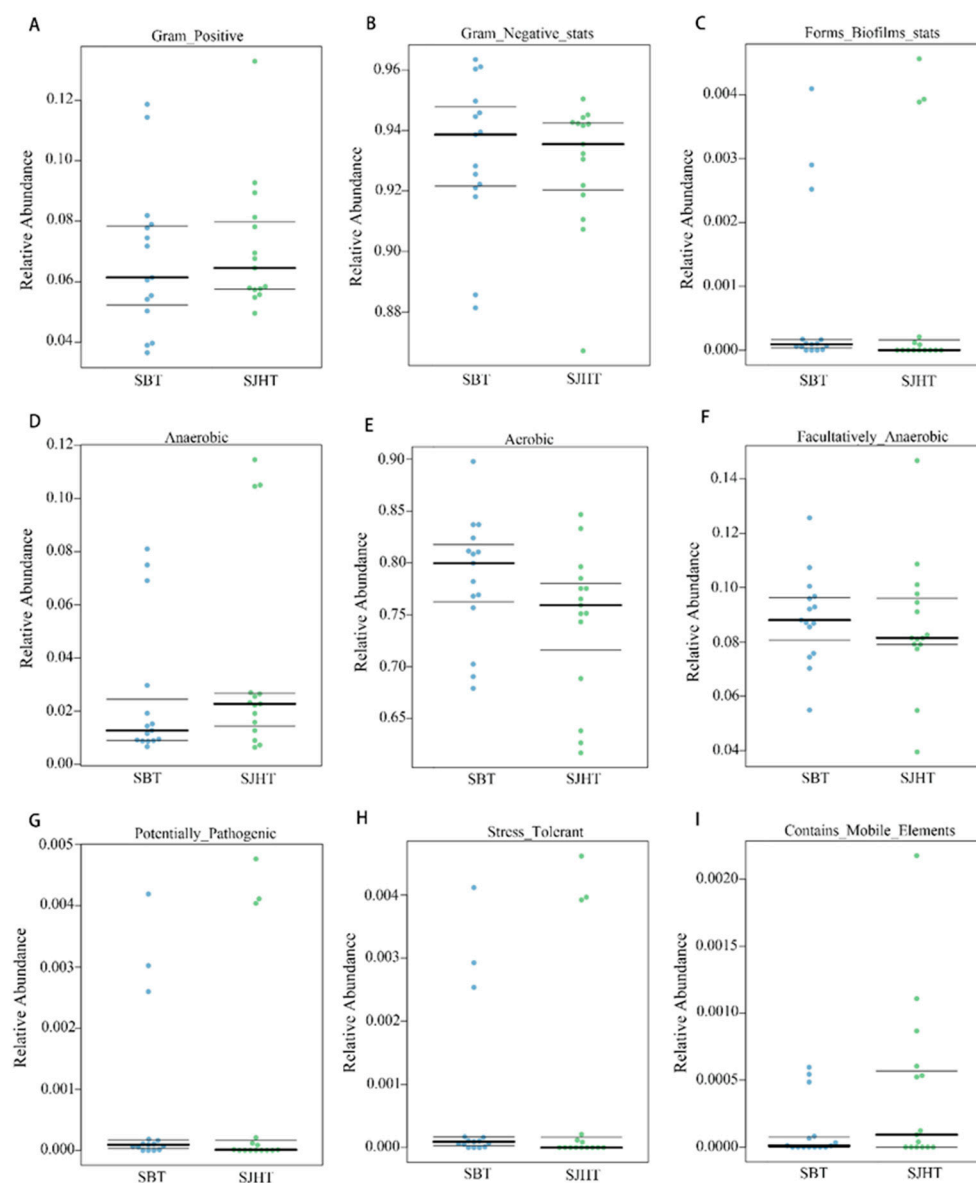


Figure 7. Relative abundance of rhizosphere soil bacterial phenotypes in *Abies ziyuanensis*. Note: Shenbaotang (SBT) and Sanjiaohutang (SJHT) refer to the sampling sites. Vertical coordinates are the relative abundance. (A) Gram_Positive; (B) Gram_Negative Stats; (C) Forms_Biofilms Stats; (D) Anaerobic; (E) Aerobic; (F) Facultatively_Anaerobic; (G) Potentially_Pathogenic; (H) Stress_Tolerant; (I) Contains_Mobile_Elements.

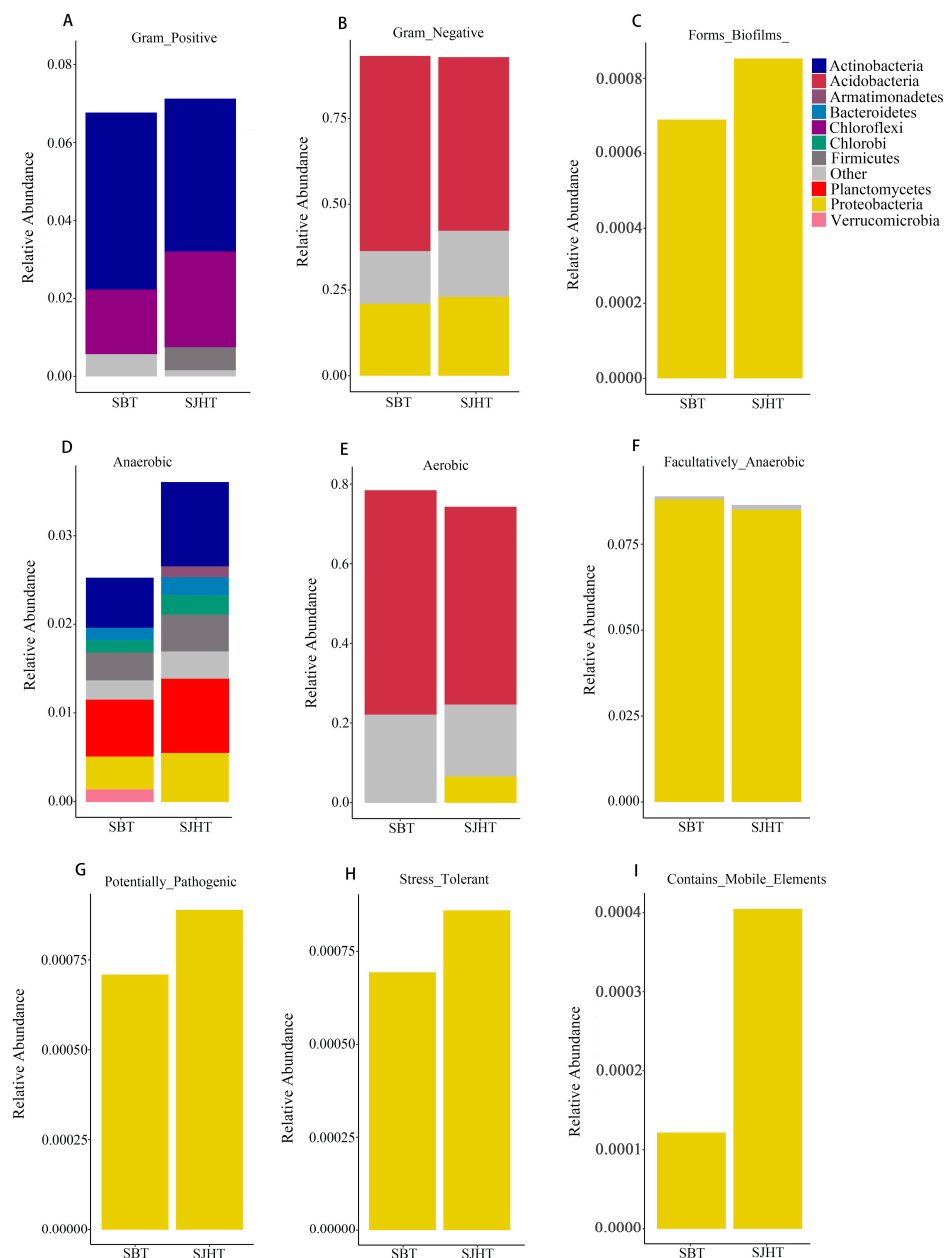


Figure 8. Species phenotype contribution of soil bacterial communities in the *Abies ziyuanensis* rhizosphere. Note: Shenbaotang (SBT) and Sanjiaohutang (SJHT) refer to the sampling sites. Vertical coordinates represent the relative abundance. (A) Gram_Positive; (B) Gram_Negative_Stats; (C) Forms_Biofilms_Stats; (D) Anaerobic; (E) Aerobic; (F) Facultatively_Anaerobic; (G) Potentially_Pathogenic; (H), Stress_Tolerant; (I) Contains_Mobile_Elements.

4. Discussion

4.1. Analysis of the Physicochemical Properties of *A. ziyuanensis* Rhizosphere Soil

Our study revealed that the majority of soil physicochemical properties exhibited no significant differences between SBT and SJHT. However, TK and AK were notably higher in SBT than in SJHT (Table 2), suggesting that although the overall soil nutrient conditions of these two sites are similar, the potassium (K) availability may play a role in distinguishing their soil environments. Similarly to the findings of this study, Li et al. [39] compared the rhizosphere soil bacterial communities of *Pygodactylus squamata* from different locations and found that TK and AN in the soil were the primary factors driving bacterial community composition at the phylum level. Furthermore, the analysis of bacterial diversity indices

revealed no significant differences between SBT and SJHT (Figure 1). This could have been because the constant root exudates of *A. ziyuanensis* (i.e., organic acids, phenols) masked the direct impact of potassium gradients on bacteria and thus maintained functional redundancy, thereby indicating that despite variations in the K content, the bacterial diversity in the rhizosphere soil of *A. ziyuanensis* was consistent across both sites. NMDS analysis further supported this conclusion, showing similar bacterial community structures in the rhizosphere soils at the two sites (Figure 2A). Bacterial communities did not cluster based on the sampling location (Figure 2B), indicating that the bacterial composition was not significantly influenced by the geographical location of the populations, suggesting that the bacterial communities in the rhizosphere soil of *A. ziyuanensis* were relatively stable and not strongly affected by minor changes in soil properties observed between SBT and SJHT. The lack of significant differences in the bacterial diversity and community structure reflects either the adaptability of bacterial communities to local environmental conditions or the presence of similar ecological niches at both locations. However, the higher K content in SBT may still have subtle effects on plant growth or bacterial activity that were not detected in this study. Future research should determine the functional roles of these bacterial communities and their potential interactions with *A. ziyuanensis* to better understand the survival of this endangered species.

4.2. Analysis of Compositional Characteristics and Soil-Driven Factors of *A. ziyuanensis* Rhizosphere Soil Bacteria

Proteobacteria, Acidobacteria, Actinobacteria, Planctomycetes, Verrucomicrobia, and Chloroflexi were the dominant bacterial phyla, accounting for 89.58 and 89.69% of the relative abundance at SBT and SJHT, respectively (Figure 3A,B). These dominant bacterial phyla play crucial roles in the root–rhizosphere ecosystem of *Abies nephrolepis*, potentially affecting nutrient cycling, organic matter decomposition, and plant–microbe interactions [40]. Proteobacteria dominate ecosystems, particularly soil ecosystems [41], and are characterized by rapid reproduction, excellent adaptability, and a widespread distribution in global soil environments. Through processes such as nitrification, denitrification, nitrogen fixation, and various organic carbon degradation pathways, they directly drove the rhizosphere C and N cycles [42]. The abundance of Proteobacteria and Acidobacteria is typically highest in soil samples [41]. Acidobacteria is a dominant phylum in the bacterial community associated with plant root zones. Nutrient-poor types are predominant and can degrade complex root exudates, such as cellulose and lignin, thereby accelerating rhizosphere carbon turnover and playing a crucial role in the C cycle of plant root zones [43]. Actinomycetes play a crucial role in organic matter cycling, plant pathogen growth inhibition in the rhizosphere, and complex polymer mixture decomposition in dead plant, animal, and fungal materials, thereby producing numerous extracellular enzymes that are beneficial for plant growth [44], and provide a chemical barrier for the *A. ziyuanensis*. Therefore, we infer that Proteobacteria and Actinobacteria are significant components of the plant rhizosphere microbial community, playing regulatory roles in the plant rhizosphere. The *A. ziyuanensis* is distributed in high-altitude areas with high humidity, low temperatures, and nutrient-poor acidic soils. The aforementioned dominant bacterial phyla all exhibit adaptive genes for acidic, low-temperature, and low-phosphorus environments. Among them, the oligotrophic strategy of the Acidobacteria phylum complements the r-strategy of the Proteobacteria phylum, jointly maintaining community stability [45]. Ji et al. [37] employed high-throughput sequencing to study five regions with different forest ages in Northeast China from June to October 2017 and found that the phylum Proteobacteria comprised the core of the microbiome of *Pinus koraiensis* root tips across all samples, regardless of the location, age, or sampling date. PCoA revealed a significant positive correlation between bacterial phyla and soil NO_3^- -N (Figure 4), suggesting that these nutrients are

key drivers in shaping the rhizosphere bacterial community structure [46]. In contrast, other soil physicochemical properties did not have significant correlations with bacterial phyla, indicating that they could selectively recruit through secondary metabolites (such as flavonoids, terpenes) in root exudates rather than inorganic nutrients. Although these soil physicochemical properties showed no significant correlation and may have had limited effects on bacterial composition, they did not strongly determine the structure of bacterial communities, though they may still play subtle roles in bacterial dynamics.

The dominant bacterial phyla in the rhizosphere of *A. ziyuanensis* maintain plant health through complementary metabolic strategies, including rapid carbon and nitrogen transformation, oligotrophic degradation, and pathogen inhibition. NO_3^- -N acts as a key environmental filter, preferentially shaping the abundance and function of the Proteobacteria phylum, while Acidobacteria and Actinobacteria maintain their dominance through chemically mediated signals from root exudates. Although phylum-level studies can reveal macroevolutionary frameworks and core functional characteristics, they also have inherent limitations. Future research should integrate family or genus-level classification for multi-layered analysis to more accurately understand microbial diversity, functions, and ecological interactions.

4.3. Analysis of Functional Groups and Phenotypic of *A. ziyuanensis* Rhizosphere Soil Bacteria

FAPROTAX functional prediction revealed that the main ecological functions of rhizosphere bacterial communities were chemoheterotrophy, aerobic chemoheterotrophy, N fixation, intracellular parasitism, and fermentation (Figure 5). Bacterial communities primarily participated in organic matter decomposition, C cycling, and N transformation processes, which are crucial for maintaining soil fertility and supporting plant growth [47,48]. The relative abundance of bacteria with functions related to the N cycle and photosynthesis was significantly lower than that of those with chemoheterotrophic functions, highlighting the importance of organic C utilization in this ecosystem. This pattern corresponds to the high-moisture, high-organic-matter acidic soil where the *A. ziyuanensis* occurs, i.e., the large input of litter provides abundant cellulose, lignin, and soluble sugars, offering sufficient carbon sources for heterotrophic microorganisms. In SBT, N fixation was significantly negatively correlated with AK, while photosynthetic functions were significantly positively correlated with AP but negatively correlated with SWC (Figure 6A). In SJHT, N fixation was negatively correlated with AK, AN, and TN but positively correlated with MBN (Figure 6B), indicating complex interactions between N-related bacterial functions and soil nutrient availability. When fast-acting nitrogen is limited, nitrogen-fixing bacteria promote microbial biomass nitrogen accumulation by releasing fixed nitrogen, thereby forming a positive feedback loop. Nitrification and nitrate reduction processes were also strongly influenced by N and *p* availability, which indicates that phosphorus is a key factor in maintaining the energy metabolism of nitrifying microorganisms and emphasizing the importance of these nutrients in shaping microbial functional dynamics. In contrast, heterotrophic functions showed no significant correlation with soil properties, indicating the lower sensitivities of these processes to changes in soil conditions. Similarly, Ji et al. found that related functions were significantly influenced by the N, *p*, and K contents of the soil habitat [37]. Therefore, under the low-nitrogen and high-potassium conditions in SBT, the addition of artificial nitrogen-fixing bacterial agents can alleviate potential nitrogen limitation. In SJHT, moderate increases in phosphorus fertilizer or litterfall phosphorus input may enhance nitrification rates.

Using BugBase to perform phenotype prediction analysis of the bacterial community in the rhizosphere soil of *A. ziyuanensis* from Yinzhua Laoshan, no significant differences in phenotypic characteristics were found between SBT and SJHT (Figure 7), indicating

that the functional potential of the rhizosphere bacterial community in this region is relatively consistent. Such consistency may have arisen from the host plant's 'functional filtering' effect on rhizosphere microorganisms; in other words, the *A. ziyuanensis* appears to prioritize the recruitment of bacterial phyla with similar metabolic strategies through stable root exudate composition (i.e., soluble sugars, phenolic acids) and a persistent acidic environment, thereby offsetting potassium gradient differences caused by microtopography. Acidobacteria, Proteobacteria, and Chloroflexi were the main contributors to the observed phenotypic characteristics. Acidobacteria and Proteobacteria were the primary contributors to the Gram-negative phenotype, and Acidobacteria also played a significant role in the aerobic phenotype (Figure 8). This is consistent with their known ecological roles, as Acidobacteria have been associated with nutrient-poor environments and use complex organic matter [49], and Proteobacteria are highly diverse and participate in various metabolic processes, including N cycling and organic matter decomposition [41]. Chloroflexi, Actinobacteria, and Firmicutes contributed to the Gram-positive phenotype (Figure 8), which is consistent with their typical cell wall structure [50–52]. The phylum Proteobacteria was a major contributor to biofilm formation, facultative anaerobic metabolism, pathogenicity, stress tolerance, and mobile element content [40], highlighting the adaptability and ecological diversity of this phylum, which can thrive under various environments and play crucial roles in both beneficial and pathogenic interactions. The relatively low contribution of the phylum Proteobacteria to pathogenic phenotypes (Figure 8) indicates that the roots of the Sitka spruce are not heavily colonized by harmful bacteria, which is beneficial for plant health. The anaerobic phenotype was supported by more bacterial phyla, including Acidobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Planctomycetes, Proteobacteria, and Verrucomicrobia (Figure 8), suggesting that anaerobic metabolic processes are distributed across multiple bacterial groups and reflecting the functional redundancy commonly observed in soil microbial communities. However, the aerobic phenotype was primarily driven by Acidobacteria and Proteobacteria, emphasizing their importance in oxygen-dependent metabolic processes. Similar studies have also shown that aerobic bacteria have the highest abundance, while anaerobic and facultative anaerobic bacteria have lower abundances. Oxidative stress-resistant bacteria reduce the potential risk of pathogens, and Gram-negative bacteria are more abundant than Gram-positive bacteria [40]. Therefore, when artificially propagating *A. ziyuanensis* seedlings, facultative anaerobic bacterial agents can be introduced to enhance root resistance.

Overall, phenotypic analysis showed that Acidobacteria, Proteobacteria, and Chloroflexi were key phyla influencing the functional potential of bacterial communities in the *A. ziyuanensis* rhizosphere. Their dominance in various phenotypic traits suggests their roles in maintaining soil health and supporting plant growth through nutrient cycling, organic matter decomposition, and stress resistance. The lack of significant differences in phenotypic lineages between SBT and SJHT further supports that bacterial communities at these sites are functionally stable and adapted to local environmental conditions. The results of this study provide valuable insights into the functional diversity and ecological roles of rhizosphere bacteria in the *A. ziyuanensis* ecosystem. Future research should explore how these bacterial phenotypes promote plant stress tolerance and ecosystem functions, particularly under environmental stressors, such as nutrient limitations or climate change. Additionally, investigating the interactions between these bacterial communities and *A. ziyuanensis* may reveal the potential of bacterial engineering to conserve and restore this endangered species.

5. Conclusions

The bacterial community in the rhizosphere soil of *A. ziyuanensis* from Yinzhong Laoshan was dominated by Proteobacteria, Acidobacteria, and Actinobacteria phyla. Among these, Proteobacteria had a significant positive correlation with soil NO_3^- -N ($R = 0.47$, $p = 0.0079$) and primarily contributed to phenotypic functions related to pathogenicity and stress tolerance. Furthermore, through functional prediction of bacterial communities at the rhizosphere of the Yinzhong Laoshan *A. ziyuanensis* using FAPROTAX, it was found that chemoheterotrophic functions predominated, followed by N cycling functions, whereas phototrophic functions were relatively weak. N cycling and phototrophic groups were significantly associated with soil physicochemical properties, but chemoheterotrophic groups showed no significant correlations. This suggests that the rhizosphere bacteria of *A. ziyuanensis* drive biogeochemical processes, such as N cycling, thereby maintaining soil functional stability. The pathogenic and stress-tolerance characteristics of dominant bacterial groups may influence host-plant adaptability. These findings provide a theoretical basis for the conservation of rhizosphere microbial resources of *A. ziyuanensis* and the directed regulation of functional bacteria in artificial seedling cultivation. Future work should (i) isolate and genome-sequence (metagenomic and metatranscriptomic) these nitrate-responsive Proteobacteria, (ii) test their plant-growth-promoting capacity under greenhouse drought and pathogen challenge, and (iii) monitor their persistence across seasonal and climatic gradients to guide scalable microbiome engineering for *A. ziyuanensis* conservation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f16091404/s1>, Figure S1: Rank-abundance curve. Note: SBT01 to SBT15 and SJHT01 to SJHT15 represent rhizosphere samples from 15 plants analyzed at the two sites, Shenbaotang (SBT) and Sanjiaohutang (SJHT); Table S1: Sequencing of rhizosphere bacteria of *Abies ziyuanensis* at different population (mean $\pm$ standard deviation, $n = 15$).

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Abbreviations

The following abbreviations are used in this manuscript:

SBT	Shenbaotang
SJHT	Sanjiaohutang

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