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Soil Type Influences Rhizosphere Bacterial Community Assemblies of Pecan Plantations, a Case Study of Eastern China

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Abstract: The rhizosphere microbiome is closely related to forest health and productivity. However, whether soil type affects pecan (*Carya illinoinensis*) rhizosphere microbiomes is unclear. We aimed to explore the diversity and structural characteristics of rhizosphere bacteria associated with pecan plantations grown in three soil types (Luvisols, Cambisols, Solonchaks) in Eastern China and analyze their potential functions through high-throughput sequencing. The results showed that the diversity and community structure of rhizosphere bacteria in pecan plantations were significantly affected by soil type and the pH, available phosphorus content, electrical conductivity, soil moisture, and ammonium nitrogen contents were the main factors. At the phylum level, the rhizosphere bacterial community composition was consistent, mainly included Actinobacteria, Proteobacteria, Acidobacteria, and Chloroflexi. At the family level, the pecan plantations formed different rhizosphere enriched biomarkers due to the influence of soil type, with functional characteristics such as plant growth promotion and soil nutrient cycling. In addition, there existed low abundance core species such as Haliangiaceae, Bryobacteraceae, and Steroidobacteraceae. They played important roles in the rhizosphere environments through their functional characteristics and community linkages. Overall, this study provides a basis for the study of the rhizosphere microbiome in different soil types of pecan plantations, and plays an important role in the sustainable management of forest soil.

Keywords: rhizosphere bacteria; *Carya illinoinensis*; soil type; community structure; community diversity; interaction network; potential functions

1. Introduction

The rhizosphere, first defined by the German scientist Lorenz Hiltner, is the region of soil affected by plant roots [1]. The rhizosphere microbiome is presented in the rhizosphere soil and can be commensal, parasitic, or mutualistic [2]. At the soil interface, the rhizosphere microbiome can promote the decomposition of soil organic matter, formation of humus, and transformation of soil nutrient elements, which form the indexes for evaluating soil environmental conditions and fertility parameters [3]; at the plant interface, it can improve the plant nutrient utilization efficiency and growth ability by regulating hormone levels, increasing nutrient absorption and inducing stress resistance [4]. Therefore, the rhizosphere microbiome plays an important role in improving the soil environment and plant productivity. There are a large number and variety of groups of bacteria in the rhizosphere microbiome [5]; therefore, bacteria occupy a dominant position in the rhizosphere that reflects the stability of the microbial community and the quality of soil to a certain extent. Therefore, research on rhizosphere bacteria is important for the reconstruction of rhizosphere microbiomes.

The soil environment is diverse, and the factors affecting its composition are complex, so there are great differences in physical and chemical properties at the macro- and microscales. As the place for the activity and multiplication, soil is the key factor in determining the composition of the rhizosphere microbiome [6–8]. Different soil types are

thought to foster specific microbial communities [9], which differ in composition and diversity. Soil factors such as soil texture are an important driving force affecting rhizosphere microbial structure, and higher soil heterogeneity and suitable moisture condition will reduce the competitive pressure within microbiomes [10], thus improving diversity; pH and nutrient levels can affect the soil environment and plant physiological characteristics, influencing the composition of rhizosphere depositions, which include root exudates [11], and changing the community composition of the rhizosphere microbiome. Therefore, understanding the influence of the soil environment on the microbial community based on the interaction mechanisms of plant–rhizosphere microbiome–soil is advantageous to the development of beneficial microbiome resources and the regulation of rhizosphere microbial composition to synergistically promote plant growth and soil ecosystem stability.

Pecan (*Carya illinoinensis* (Wangenh.) K. Koch), a member of the hickory genus (*Carya* spp., Juglandaceae), is an economically valuable forest tree with high comprehensive utilization value, integrating fruit, oil, wood, and afforestation. Pecan is native to North America, with a natural distribution ranging between approximately 30° N and 42° N [12,13]. Pecan was first introduced into China in the early 20th century and is now mainly concentrated in the Yangtze River Basin and eastern subtropical regions, including Yunnan and Jiangsu Provinces [14]. In recent years, pecan has reached an increasingly status in the forestry industry, but production still faces some problems such as the bottleneck of cultivation technology and the excessive use of chemical fertilizers. The utilization of rhizosphere microbial resources is one of the effective ways to realize the sustainable development of the pecan industry, and the study of the changes in the biological characteristics of rhizosphere microbiota under different soil conditions is the first step to improve the adaptability and utilization of microbial resources. At present, research on the microbiome of pecan is mainly focused on biological control [15] and morphological identification [16,17], and an overall understanding of the rhizosphere microbiome, including diversity and community composition, is lacking.

In this study, based on high-throughput sequencing technology, we studied the community structure and diversity of rhizosphere bacteria in soils associated with 7-year-old pecan plantations (Pawnee) grown in three soil types in Jiangsu Province, Eastern China. We addressed the following scientific questions:

I.   Are the diversity and community composition of rhizosphere bacteria in pecan plantations affected by soil type?
II.   What are the compositions and biomarkers of the rhizosphere bacterial communities with pecan plantations grown in three soil types?
III.   What are the main soil factors driving the rhizosphere bacterial community of pecan plantations?
IV.   What are the main functional types of rhizosphere bacteria in pecan plantations?

2. Materials and Methods

2.1. Study Area

Due to the latitudinal zonality of the soil distribution, we selected the main pecan production areas in southern, central, and northern Jiangsu Province, Eastern China which are located in Jurong (31°52′47″ N, 119°9′5″ E), Taizhou (32°27′25″ N, 120°3′48″ E), and Yancheng (33°1′14″ N, 120°46′33″ E), respectively. In the three sampling areas, the mean annual temperature (MAT) is 15.6 °C, 14.8 °C and 14.1 °C with mean annual precipitation (MAP) values of 1018.6 mm, 1037.7 mm, and 1058.4 mm, respectively. These areas are located in the subtropical monsoon climate zone, with high temperatures and rainy conditions in summer, low temperatures and rainless conditions in winter, and obvious monsoon climate characteristics. According to the FAO World Reference Base (WRB) for Soil Resources (IUSS Working Group, 2014), the soil types are Luvisols (Jurong), Cambisols (Taizhou), Solonchaks (Yancheng). In the study areas, there is a pure plantation forest of 7-year-old Pawnee. The planting density is 6 m × 8 m, and the management measures

were consistent among the study areas. We surveyed pecan growth and soil characteristics before sampling, and the results are shown in Table 1.

Table 1. Basic information of sampling sites in different soil types in Jiangsu Province, Eastern China.

Soil Type	Sampling Area	Altitude (m)	Soil Texture	Soil Bulk Density (g·cm^{-3})	Soil Porosity (%)	Average Tree Height (m)	Average Diameter at Breast Height (cm)	Average Crown (m)
Luvisols	Jurong	24.21	Loam	1.39~1.50	44~48	7.47	9.21	3.95
Cambisols	Taizhou	7.85	Sandy Loam	1.31~1.46	45~51	7.21	9.76	4.03
Solonchaks	Yancheng	3.26	Silt Loam	1.12~1.42	46~58	6.23	8.53	3.79

Soil texture classification references the FAO World Reference Base (WRB) for Soil Resources.

2.2. Experimental Design and Sample Collection

We used a single-factor completely randomized design in this experiment and collected rhizosphere samples from the three soil types of pecan plantations in the above sampling areas, with six biological replicates for each soil type. Moreover, to ensure the representativeness of the samples and reduce the within-group error caused by other environmental factors, we controlled the sampling time within one month and set four sampling points for each tree following the principle of multi-draw sampling; the rhizosphere soil samples from three trees were then mixed into one sample to form one replicate. Rhizosphere soil was collected following the method described by Riley et al. [18,19]. A total of 54 healthy sample trees of the same age and uniform growth were randomly selected from the three regions to obtain 18 rhizosphere soil samples.

We collected the rhizosphere soil samples in the above three sampling sites from the end of September to the beginning of October 2020. The specific sampling steps were as follows: we established a radius of 0.5 m from the base of the trunk and sampling points to the east, south, west, and north, and samples were collected at depths of 0–60 cm from the soil surface. We used an earth drill to sample the soil and collected fine roots with diameters of less than 2 mm from the soil. First, we shook off and removed the large amounts of soil not adhered to roots; and then, with a sterile brush, we collected the soil that was still adhered to the roots (<5 mm). The soil from the four sampling points around three trees was mixed to form one rhizosphere soil sample. The soil samples were placed in sterile bags, stored in foam containers with dry ice, and transported to the laboratory. In the laboratory, we sieved the soil samples through 2 mm meshes to remove stones and debris. The rhizosphere soil samples were divided into two parts: one part of the fresh soil samples was stored at −80 °C for rhizosphere soil microbial analysis and determination of soil ammonium and nitrate nitrogen contents, and the other part was used for determination of residual soil properties after natural air-drying.

2.3. Soil Property Determination

Soil pH was estimated from a 1:2.5 soil:water (*w/v*) mixture using a pH meter (pH 700, Eutech, San Francisco, CA, USA), and electrical conductivity (EC) was estimated from a 1:5 soil:water (*w/v*) mixture using a conductivity metre (DDS-307A, Rex Electric Chemical, Shanghai, China). Soil moisture (SM) content was measured at 105 °C overnight by the oven drying method. Total carbon (TC) and total nitrogen (TN) contents were measured with an elemental analyzer (2400 Series II CHNS/O, PerkinElmer, Waltham, MA, USA) according to the Dumas combustion method [20]. Soil samples were extracted with 2 mol·L^{-1} potassium chloride (KCL) solution, and ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) contents were measured by using the indophenol blue colorimetric method and dual-wavelength colorimetric method (wavelength of 225 nm and 275 nm) after extraction [21]. The available phosphorus (AP) content was analyzed by the molybdenum antimony colorimetric method after ammonium bicarbonate ($NaHCO_3$) extraction. The available potassium (AK) content was analyzed by flame photometry after ammonium

acetate (CH_3COONH_4) extraction. The soil samples were digested by a mixture of nitric-perchloric-hydrofluoric acid (HNO_3-$HClO_4$-HF), and the total phosphorus (TP) and total potassium (TK) contents were determined by molybdenum antimony colorimetric and flame photometry, respectively, after extraction. The $NH4^+$-N, $NO3^-$-N, AP, and TP contents were determined by ultraviolet spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan), TK and AK were determined by flame spectrophotometry (BWB XP, BWB Technologies, Newbury, UK) [22].

2.4. Soil DNA Extraction and PCR Amplification

Approximately 0.5 g of fresh soil was used for microbial DNA extraction, which was performed with the FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The DNA quality was determined by 1% agarose gel electrophoresis (5 V/cm, 20 min), and the DNA concentration and purity were checked on a NanoDrop2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

The extracted DNA was used as a template for PCR amplification, and the hypervariable V3-V4 region of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-ACTCCTACGGGAGGCAGCAG-3′) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, Carlsbad, CA, USA). The PCR was performed with a TransGen AP221-02: *TransStart* Fastpfu DNA polymerase in a 20 μL reaction system that included 5× TransStart FastPfu buffer (4 μL), 2.5 mM dNTPs (2 μL), forward primer (5 μM; 0.8 μL), reverse primer (5 μM; 0.8 μL), *TransStart* FastPfu DNA Polymerase (0.4 μL), template DNA (10 ng), and ddH_2O (up to 20 μL). The reaction conditions and amplification procedures were performed as follows: initial denaturation at 95 °C for 3 min; 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s; and a single extension at 72 °C for 10 min, ending at 10 °C. PCRs were performed in triplicate. The PCR product was extracted from a 2% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using a Quantus™ Fluorometer (Promega, Madison, WI, USA) at the end of amplification.

2.5. Illumina MiSeq Sequencing

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, CA, USA) according the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA777007).

2.6. Processing of Sequencing Data

The raw sequencing data from sequencing were demultiplexed, quality-filtered by fastp (HaploX, Shenzhen, China, version 0.20.0), and merged by FLASH (version 1.2.7) to obtain effective sequencing tags.

UPARSE software (version 7.1) was used to perform operational taxonomic unit (OTU) clustering and remove chimeras at a similarity of 97%. The taxonomy of each OTU representative sequence was analyzed by the RDP classifier (version 2.2) based on comparison with the Silva bacterial database (release 132; confidence threshold of 0.7). A total of 505,134 effective sequences and 10,800 OTUs were obtained after clustering and standardizing of data to the minimum sample sequence number (28063), and the subsequent analysis was conducted based on the data after homogenization.

Alpha diversity analyses: Mothur (version 1.30.2) software was used to calculate the α-diversity indexes, including community richness (Sobs, Chao, and Ace indexes), diversity (Shannon and Simpson indexes), and coverage. R software (version 3.4.2, Vienna, Austria) was used to generate rarefaction curves. The rarefaction curves of rhizosphere bacteria indicated that the standardization process did not cause a large amount of data loss, and

the number of sequences and sequencing depth could accurately reflect the rhizosphere bacterial community of pecan plantations.

Beta diversity analyses: QIIME software (version 1.9.1) was used for hierarchical clustering analysis based on the unweighted UniFrac distance algorithm, and a hierarchical clustering tree was drawn in R software (version 3.4.2). Nonmetric multidimensional scaling analysis (NMDS) based on the unweighted UniFrac distance algorithm and analysis of similarity (ANOSIM) was performed by the R package vegan (version 3.4.2).

Community composition analysis: R software (version 3.4.2) was used to draw a histogram of community abundance at the phylum and family levels (others < 0.01). Microbial differential discriminant analysis (LDA > 3.5) at the phylum to family levels was performed by LEfSe software (Galaxy version 1.0). Single-factor network analysis was performed with Networkx (version 2.1) software ($R \geq 0.5$, $p < 0.05$).

Analysis of soil environmental factors: The variance inflation factor (VIF) was used to analyze soil environmental factor data and remove invalid indicators (VIF > 10). A Mantel test was performed by QIIME software (version 1.9.1) after applying the unweighted UniFrac distance algorithm to microbial data and the Bray-Curtis distance algorithm to soil environmental factor data. SPSS software (version 24.0) was used to perform Pearson correlations between the α-diversity indexes and soil factor data. RDA of microbial and soil properties was performed with the R package vegan (version 3.4.2).

Bacterial function prediction: To compare the data to KEGG databases, functional prediction of bacteria was performed by PICRUSt2 (version 2.4.1), and KEGG orthology (KO) and KEGG pathway results were obtained.

The α-diversity indexes and soil factor data of rhizosphere samples from different soil types were compared by one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) post hoc test in SPSS software (Version 24.0). All the data for the tables were organized and tables were created with Microsoft Excel 2010 (Microsoft, Redmond, WA, USA).

3. Results

3.1. Differences in Rhizosphere Soil Properties

There were significant differences in the rhizosphere soil properties among the three soil types (Table 2). The EC, pH, and NO_3^--N contents in the Luvisols were significantly lower than those in the other two soil types, while the NH_4^+-N content was significantly higher in the Luvisols than in the other soil types. The AP and TP contents were highest in the Cambisols, while the SM, TC, and TK contents were highest in the Solonchaks. The TN and AK contents showed no significant differences among the rhizosphere samples.

3.2. The α-Diversity Analysis

The values of the α-diversity indexes of the rhizosphere bacteria were shown in Table 3. The values of the Sobs, Shannon, Simpson, and Chao indexes indicated that the bacterial diversity was highest in the Cambisols, followed by the Solonchaks and the Luvisols. The Sobs index was significantly lower for the Luvisols than for the Cambisols (15.21%) and Solonchaks (11.27%). The Ace index was lowest in the Luvisols, but higher in the Solonchaks than in the Cambisols. We speculated that the Ace index increased because of the growth of dominant rhizosphere bacteria in the Solonchaks.

Table 2. Analysis of rhizosphere soil environmental factors in pecan plantations.

Soil Type	EC (μS·cm^{-1})	pH	SM (%)	TC (g·kg^{-1})	TN (g·kg^{-1})	NH$_4^+$-N (mg·kg^{-1})	NO$_3^-$-N (mg·kg^{-1})	AP (mg·kg^{-1})	AK (mg·kg^{-1})	TP (g·kg^{-1})	TK (g·kg^{-1})
Luvisols	58.03 ± 18.74 b	6.49 ± 0.64 b	11.99 ± 1.39 b	11.97 ± 1.78 b	1.15 ± 0.62 a	10.00 ± 1.09 a	3.68 ± 0.69 b	43.73 ± 8.28 b	185.08 ± 68.41 a	1.29 ± 0.08 c	10.03 ± 0.63 b
Cambisols	221.75 ± 84.20 a	7.36 ± 0.07 a	16.54 ± 2.66 b	12.12 ± 2.09 b	1.32 ± 0.89 a	6.73 ± 2.22 b	21.69 ± 14.63 a	393.28 ± 123.97 a	133.19 ± 35.15 a	5.45 ± 0.31 a	10.38 ± 0.45 b
Solonchaks	268.42 ± 64.63 a	7.76 ± 0.06 a	26.92 ± 6.23 a	16.38 ± 0.97 a	1.28 ± 0.40 a	5.45 ± 0.63 b	39.32 ± 16.48 a	72.23 ± 7.47 b	186.61 ± 20.14 a	1.60 ± 0.07 b	14.24 ± 4.38 a

Different letters indicate significant difference of rhizosphere soil samples ($p < 0.05$).

Table 3. Alpha diversity indexes of rhizosphere bacteria in different soil types of pecan plantations.

Soil Type	Sobs	Shannon	Simpson	Ace	Chao	Coverage
Luvisols	3122.83 ± 191.28 b	6.8481 ± 0.0997 b	0.0026 ± 0.0003 a	5048.02 ± 666.50 a	4516.73 ± 294.67 b	0.9582 ± 0.0034 a
Cambisols	3597.83 ± 94.47 a	7.0267 ± 0.0692 a	0.0024 ± 0.0007 a	5236.60 ± 169.37 a	5207.75 ± 150.89 a	0.9511 ± 0.0016 b
Solonchaks	3474.83 ± 119.76 a	7.0227 ± 0.0656 a	0.0021 ± 0.0002 a	5304.12 ± 580.52 a	5049.36 ± 195.47 a	0.9532 ± 0.0024 b

Different letters indicate significant difference of rhizosphere soil type ($p < 0.05$).

In general, there were significant differences in the rhizosphere bacterial α-diversity in the different pecan soil types, indicating that bacteria were more abundant in the Cambisols than in the Solonchaks and Luvisols. In addition, the coverage index of the rhizosphere samples was above 0.95 and this finding suggested that the sequencing analysis in the study was suitable.

3.3. Compositions of the Rhizosphere Bacterial Communities

There were 45 phyla and 612 family bacterial groups were identified in the rhizosphere soils of pecan. A histogram of community abundance at the phylum and family levels was made to reflect the dominance and relative abundance ratios of the rhizosphere bacteria (Figure 1).

At the phylum level, the rhizosphere bacteria were mainly composed of Actinobacteria (mean: 25.29%), Proteobacteria (mean: 22.84%), Acidobacteria (mean: 15.15%), and Chloroflexi (mean: 13.10%), accounting for more than 76% of the total abundance. In addition, the relative abundance of Firmicutes was greater than 10% only in the Cambisols, and this value was higher than that in the other soils (Figure 1a).

At the family level, the rhizosphere bacteria were mainly composed of Vicinamibacteraceae (mean 3.90%), norank_o_Vicinamibacterales (mean 4.44%), Xanthobacteraceae (mean 3.06%), Bacillaceae (mean 2.89%), norank_o_Gaiellales (mean 2.81%), Roseiflexaceae (mean 2.70%), and Nocardioidaceae (mean 2.70%), accounting for more than 22% of the total abundance (Figure 1b).

3.4. Differences in Rhizosphere Bacterial Community Structure

The results of the rhizosphere bacterial β-diversity analysis are shown in Figure 2. The clustering results showed that the 18 rhizosphere soil samples were well divided into three groups corresponding to the three soil types and that the samples from the Cambisols and Solonchaks were grouped together (Figure 2a). NMDS and ANOSIM showed that there were significant differences in the community compositions of rhizosphere bacteria in the three soil types (ANOSIM, $R = 0.8679$, $p = 0.001$); the sample points from the Cambisols and Solonchaks were close to each other, while those from the Luvisols were far away from them (Figure 2b, NMDS, stress = 0.092). In conclusion, at the OTU level, the compositions and structures of rhizosphere bacteria were similar between the Cambisols and Solonchaks but significantly different from those in the Luvisols.

The LEfSe results of the bacteria from the phylum level to the family level are shown in Figure 3. There were 6 phyla and 36 families (LDA > 3.5) in the rhizosphere samples, with significant enrichment differences.

To further determine the importance of the bacterial taxa in the rhizosphere environment, a single-factor network analysis was performed at the family level. In the rhizosphere network, there were 234 positive correlations and 189 negative correlations within the bacterial communities (Figure 4).
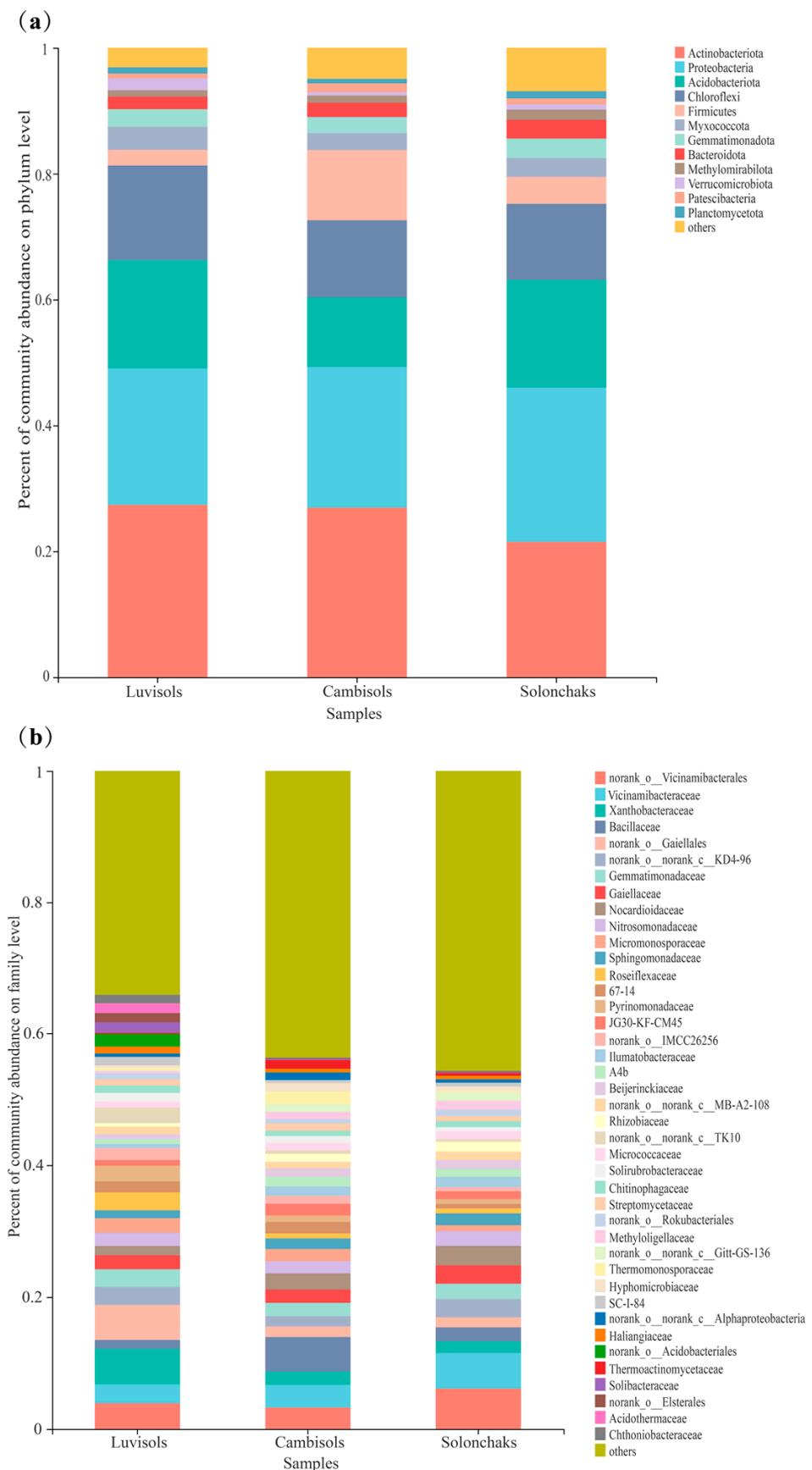
Figure 1. Taxonomic level abundance of phylum (**a**) and family (**b**) of rhizosphere bacteria in different soil types of pecan plantations.
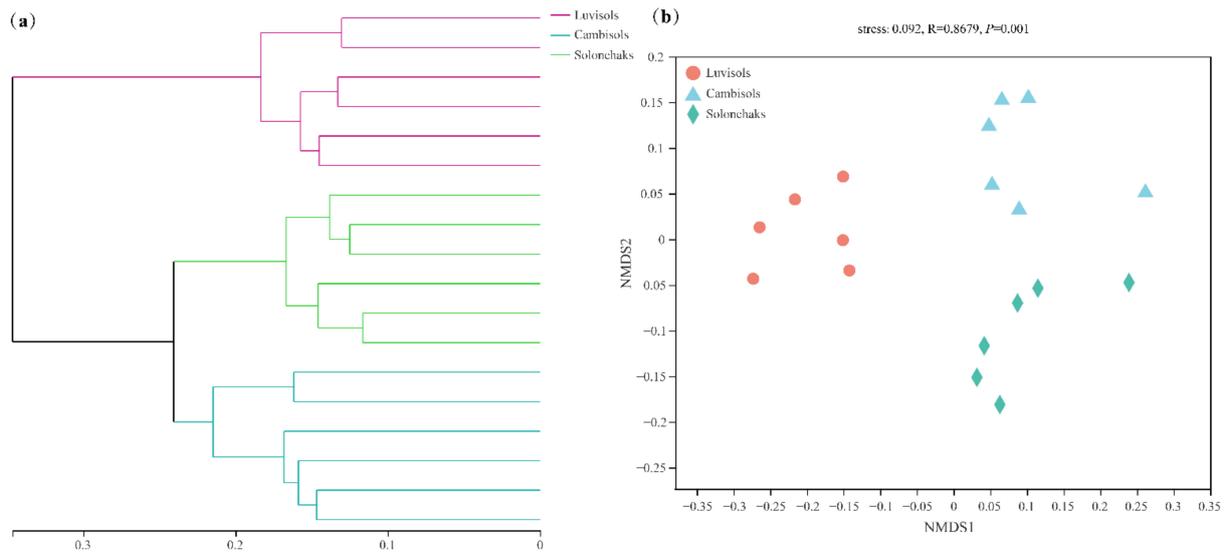
Figure 2. Beta diversity analysis of rhizosphere bacteria in different soil types of pecan plantations. (**a**) Hierarchical clustering tree represents the distance of sample branches. (**b**) NMDS and ANOSIM reflect differences and distances of samples. The stress value is used to test the quality of NMDS analysis results, and the ranking of results is better when stress <0.1. The R and *p* values represent the results of ANOSIM analysis.
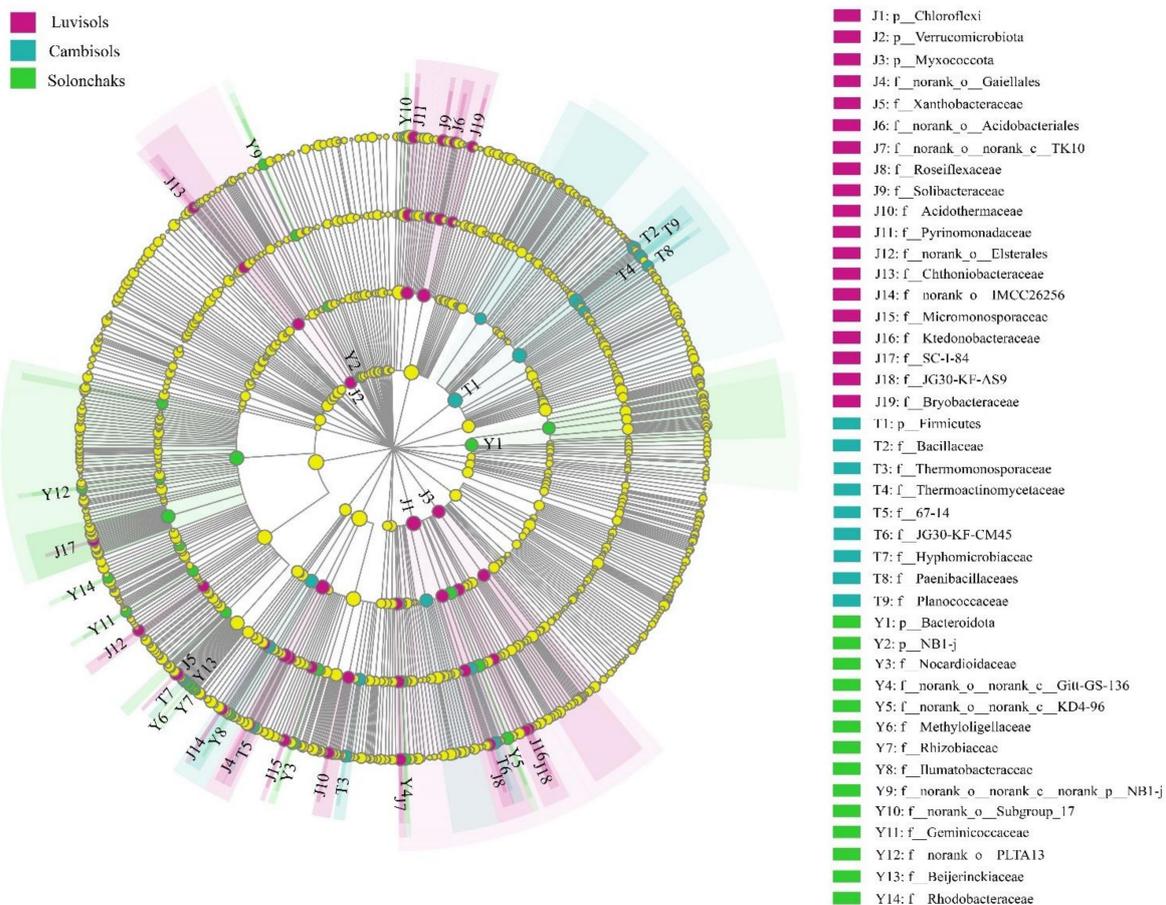

Figure 3. Community difference of LEfSe analysis of rhizosphere bacteria in different soil types of pecan plantations. The LEfSe analysis shows the top 50 species in abundance. Different node colors indicate that microbial species are enriched and significant differences in corresponding groups, while light yellow nodes indicate no significant difference.
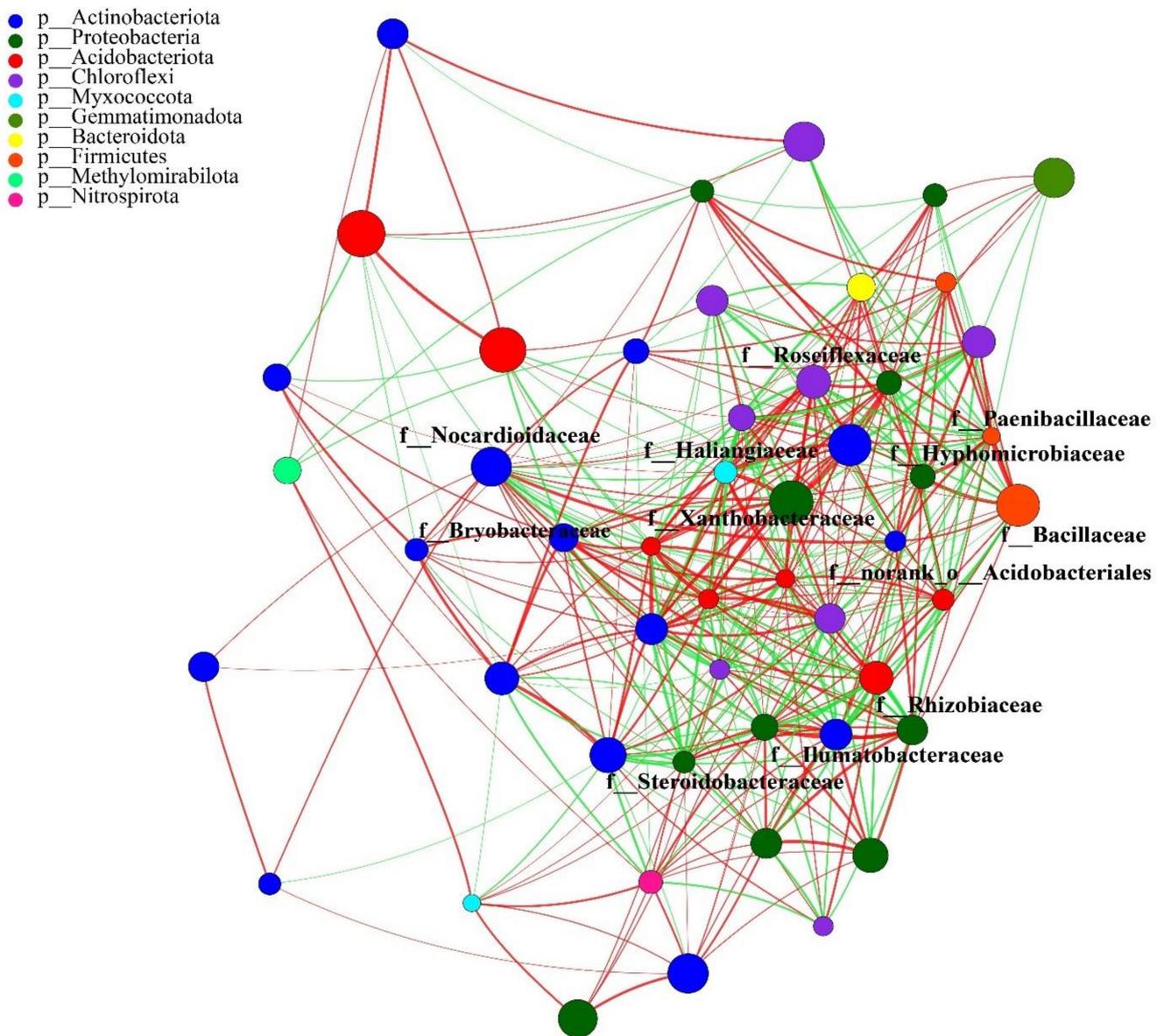
Transitivity=0.62　Diameter=4　Average shortest path length=1.77

Figure 4. Rhizosphere bacterial ecological network in different soil types of pecan plantations. The network shows the top 50 species in abundance. Node size indicates species abundance, and different colors indicate different species. Red on the line indicates positive correlation and green indicates negative correlation. The thickness of the line indicates the magnitude of the correlation coefficient.

The core rhizosphere biomarkers, which are from the comprehensive evaluation of degree centrality (DC), closeness centrality (CC), and betweenness centrality (BC) and play important roles in the rhizosphere network environment, were characterized as the bacteria with significant rhizosphere enrichment characteristics in the LEfSe analysis, and the results are shown in Table 4. In addition, some taxa, such as Haliangiaceae (DC = 0.49, CC = 0.64, BC = 0.03), Bryobacteraceae (DC = 0.49, CC = 0.64, BC = 0.02), and Steroidobacteraceae (DC = 0.49, CC = 0.64, BC = 0.04) were not significantly enriched, but had a high number of connected nodes and comprehensive parameter values in the rhizosphere network that were showed closely linked with other microbiomes. These were also considered important core taxa.

Table 4. Properties of rhizosphere bacterial biomarkers in different soil types of pecan plantations (corresponding to Figures 3 and 4.).

Phylum	Family	Group	LDA	Degree	Clustering	Degree Centrality	Closeness Centrality	Betweenness Centrality
Proteobacteria	Xanthobacteraceae	Luvisols	4.27	24	0.66	0.49	0.65	0.01
Acidobacteriota	norank_o__Acidobacteriales	Luvisols	4.02	25	0.66	0.51	0.66	0.02
Chloroflexi	Roseiflexaceae	Luvisols	3.92	24	0.64	0.49	0.64	0.02
Firmicutes	Bacillaceae	Cambisols	4.28	21	0.59	0.43	0.62	0.02
Proteobacteria	Hyphomicrobiaceae	Cambisols	3.65	25	0.60	0.49	0.65	0.02
Firmicutes	Paenibacillaceae	Cambisols	3.54	24	0.56	0.49	0.65	0.03
Actinobacteriota	Nocardioidaceae	Solonchaks	3.92	23	0.58	0.47	0.61	0.04
Proteobacteria	Rhizobiaceae	Solonchaks	3.67	22	0.68	0.45	0.62	0.01
Actinobacteriota	Ilumatobacteraceae	Solonchaks	3.66	24	0.63	0.49	0.64	0.02

The first two columns represent the taxonomic names with significant differences ($p < 0.05$). Group represents the significant enrichment of the species in this sample grouping. LDA represents the influence of species on the difference effect. Degree represents the connectivity and the number of nodes connected to nodes. Clustering indicates the connection between adjacent nodes. The larger the three values of degree centrality, closeness centrality, and betweenness centrality are, the more important the nodes are in the network.

3.5. Correlation Analysis of Rhizosphere Soil Factors and Bacteria

At the OTU level, VIF analysis (VIF = 10) was carried out for the rhizosphere soil factors. Since the VIF value of the TP was higher than 10, we removed this environmental factor, and a subsequent correlation analysis was conducted for the remaining indicators.

The results of the Mantel test showed that regional soil factors had a significant influence on the bacteria in the rhizosphere environment ($R = 0.747$, $p = 0.001$). Therefore, Pearson correlation analysis was conducted between rhizosphere soil factors and bacterial α-diversity indexes for the different soil types (Table 5). The EC, pH, and NO_3^--N contents were significantly positively correlated with the Sobs, Shannon, and Chao indexes, while the NH_4^+-N content was significantly negatively correlated with these indexes. The SM and AP contents were significantly positively correlated with the Chao index. There was no significant correlation between other environmental factors and diversity indexes.

Table 5. Correlation analysis between rhizosphere soil factors and bacterial α-diversity indexes.

Index	EC	pH	SM	TC	TN	NH_4^+-N	NO_3^--N	AP	AK	TK
Sobs	0.733 **	0.788 **	0.463	0.226	0.203	−0.653 **	0.485 *	0.561 *	−0.187	0.132
Shannon	0.724 **	0.812 **	0.535 *	0.352	0.144	−0.657 **	0.480*	0.330	−0.054	0.200
Simpson	−0.500 *	−0.492 *	−0.352	−0.193	0.125	0.256	−0.415	0.280	−0.027	−0.277
Ace	0.337	0.515 *	0.269	0.077	0.252	−0.194	0.294	0.061	0.026	0.286
Chao	0.750 **	0.781 **	0.484 *	0.190	0.163	−0.638 **	0.528 *	0.567 *	−0.229	0.279
Coverage	−0.722 **	−0.747 **	−0.418	−0.111	−0.170	0.578 *	−0.512 *	−0.563 *	0.254	−0.175

* Represents significant correlation ($p < 0.05$), ** represents extremely significant correlation ($p < 0.01$).

RDA of rhizosphere environmental factors and bacteria is shown in Figure 5. The first two RDA axes explained 66.89% of the variation in the data, with RDA axis 1 accounting for 43.48% and RDA axis 2 accounting for 23.41%. The pH ($r^2 = 0.869$, $p = 0.001$), AP content ($r^2 = 0.815$, $p = 0.001$), EC ($r^2 = 0.781$, $p = 0.001$), SM content ($r^2 = 0.691$, $p = 0.001$) and NH_4^+-N content ($r^2 = 0.683$, $p = 0.001$) were the main soil factors affecting the community structures of rhizosphere bacteria, and pH was the primary factor, having a significant correlation.
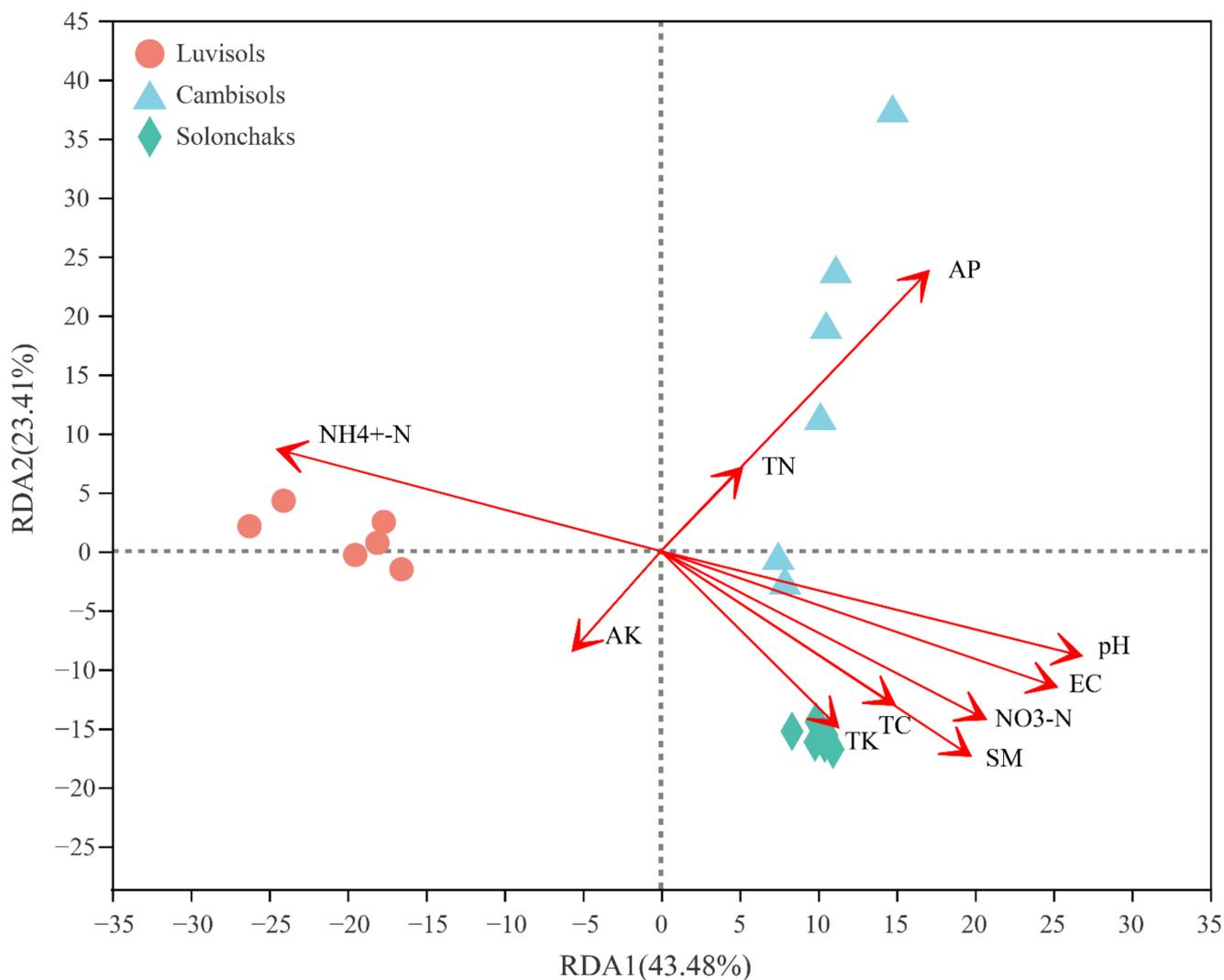
Figure 5. RDA analysis of rhizosphere soil factors and bacteria in pecan plantations.

3.6. Functional Prediction of Rhizosphere Bacteria

PICRUSt2 function prediction was performed on the rhizosphere bacterial community, and functional information related to the rhizosphere bacteria was obtained by comparison with the KEGG database, as shown in Figure 6.

In the pathway-level functional heatmap, a total of 6 types of level 1 functional biological metabolic pathways were identified: metabolism, genetic information processing, environmental information processing, cellular processes, human diseases, and organismal systems. Metabolism was the main function of rhizosphere bacteria, accounting for more than 78% of the functional roles on average (Figure 6a). A total of 46 sub-functions were identified at the secondary functional level. Global and overview maps (mean 40.59%), carbohydrate metabolism (mean 9.24%), amino acid metabolism (mean 8.14%), and energy metabolism (mean 4.49%), which belong to the metabolic pathway, were the main secondary functions (Figure 6b).
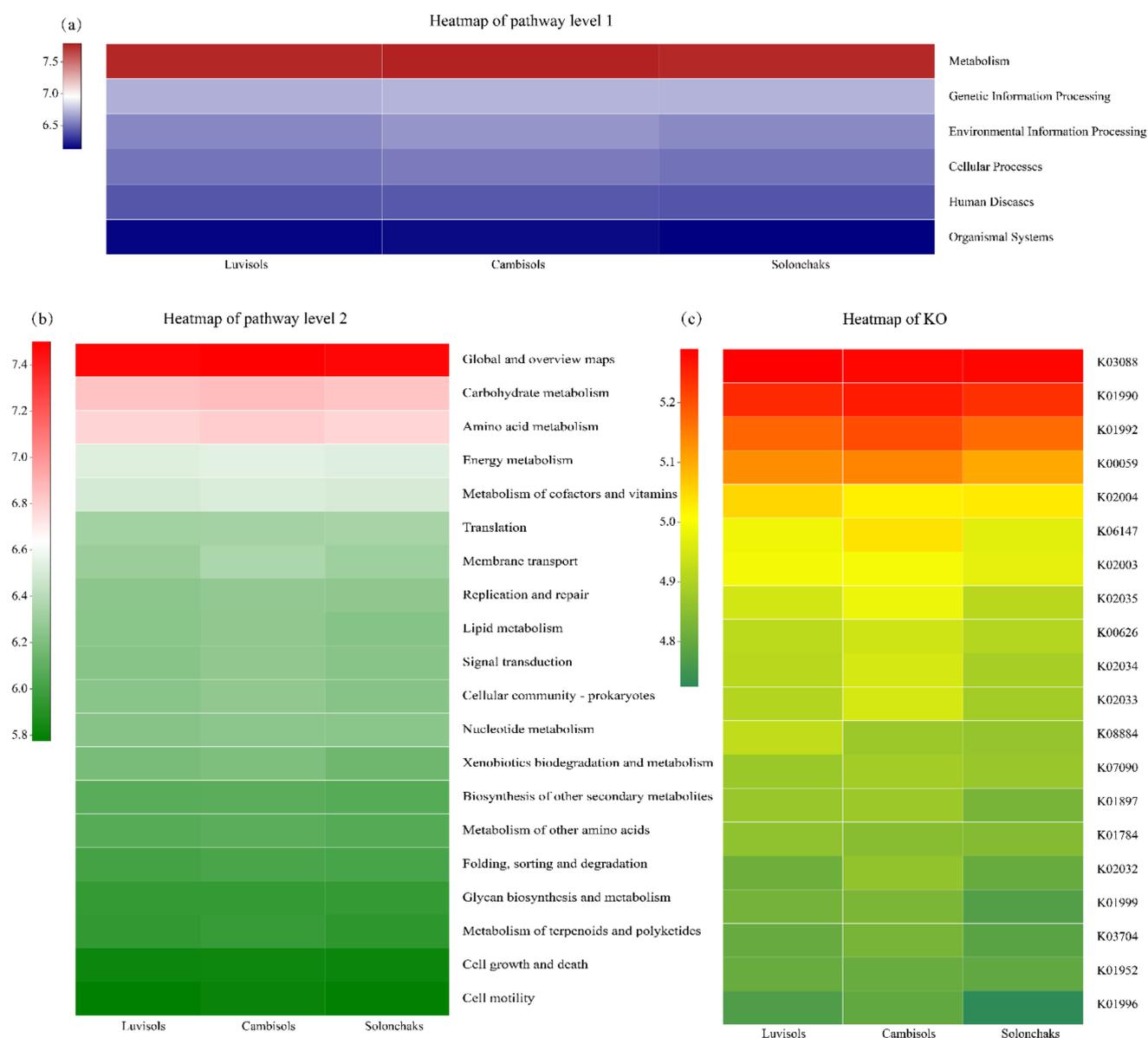
Figure 6. Prediction of rhizosphere bacterial function in different soil types of pecan plantations. The functional heatmaps show the top 20 functional types in abundance, and the color depth indicates the abundance size.

In the functional heatmap of the KO, K03088, K01990, K01992, K00059 and K02004 were the main KO functional types enriched in the rhizosphere bacteria, with average relative abundances of 0.54%, 0.49%, 0.43%, 0.37%, and 0.30%, respectively (Figure 6c). K03088 (RNA polymerase sigma-70 factor, ECF subfamily) has been proven to respond to changes in the external environment by regulating the transcription of genes related to biological or abiotic stressors and improving the adaptability of bacteria [23]. K01990 (ABC-2 type transport system ATP-binding protein), K01992 (ABC-2 type transport system permease protein), and K02004 (putative ABC transport system permease protein) are members of the ABC transporter family, which not only participate in the transport of plant hormones, ions, and secondary metabolites, but also facilitate an increase in resistance [24], and play important roles in maintaining cell stability. K00059 (3-oxoacyl-[acyl-carrier protein] reductase) can participate in lipid metabolic processes and is closely related to the biosynthesis of fatty acids and unsaturated fatty acids [25].

In conclusion, among the three soil types, the predicted gene copy numbers of pecan rhizosphere bacteria were highest in the Cambisols, followed by the Solonchaks and Luvisols. Comparison with the KEGG database demonstrated that the biological metabolic pathways of the rhizosphere bacteria were composed of 6 level 1 functional layers, mainly related to metabolism, genetic information processing, and environmental information processing, and 46 level 2 functional layers, mainly related to global and overview maps, carbohydrate metabolism and amino acid metabolism. The KO functional types included K03088, K01990, K01992, K00059, K02004 among others. These functional types reflected the richness of the rhizosphere bacterial functions in pecan plantations.

4. Discussion

4.1. Effects of Different Soil Environments on the Rhizosphere Bacterial Diversity and Community Structure of Pecan Plantations

It is generally believed that the interactions between plants and soil shape the microbial community structures [26]. The soil environment harbors microbiomes, and abiotic factors are the main factors driving the compositions of microbial communities [27]. The rhizosphere is the region in which microbiomes are most active, so the diversity and community compositions of rhizosphere microbiome are directly affected by soil properties. In this study, there were significant differences in rhizosphere bacterial diversity and community structure under different soil environments. The α-diversity of rhizosphere bacteria was highest in the Cambisols, followed by the Solonchaks and the Luvisols. The β-diversity analysis showed that the rhizosphere bacterial community structure was relatively similar between the Cambisols and Solonchaks and that the community structures in these soil types were significantly different from that in the Luvisols. The physical structure of soil, especially high soil porosity, is conducive to microbial reproduction and growth [28]. The soil bulk density of Cambisols ranges from 1.31 g·cm^{-3} to 1.46 g·cm^{-3}, and the porosity is greater than 45%. This good soil structure and excellent water permeability provide enough space for the growth of rhizosphere bacteria. The RDA analysis showed that the pH level and the AP, EC, SM, and NH4$^+$-N contents were the main environmental factors affecting the structure of the rhizosphere bacterial community. In previous studies, soil pH and nutrient status were both shown to impact the compositions of microbial communities [29]. However, because there is an optimal pH value for microbial growth, the tolerance range of microbiomes to pH changes is small. Therefore, when pH changes to a large degree, its effect will gradually increase, and pH will become the primary influencing factor [30], consistent with the results of this study. Nitrogen and phosphorus are important elements for soil nutrient cycling and plant growth, and have effects on nutrient metabolism in the rhizosphere microbiome and regulating microbial diversity and community structure [31]. Soil moisture was another important factor and could improve the nutrient acquisition and motility of microbiome [32]. Meanwhile, the research showed that bacteria were more sensitive than fungi to changes in SM content [33]. Therefore, we speculated that the differences in the diversity of rhizosphere bacteria in the three soil types was the result of the comprehensive effects of soil properties. The good soil physical structure and the more favorable moisture and nutrient conditions in the Cambisols allowed them to support the highest number of taxa and greatest microbiome diversity. Moreover, the differences in rhizosphere soil factors could also explain the differences in the community structures of the samples. The pH was the primary factor affecting the composition of rhizosphere bacteria; Cambisols and Solonchaks are alkaline soils (pH > 7), differing significantly from the acidic soil in the Luvisols. In addition, our results showed that the EC, pH, SM, and AP contents were positively correlated with indexes such as the Sobs and Shannon indexes, while the NH4$^+$-N content was mostly negatively correlated with the indexes. This result was different from the results reported by Huang et al. [34]. Different forms of nitrogen have significant impacts on the pH of the plant root environment [35]; alkaline medium is conducive to the absorption of NH4$^+$ by plant roots and reduces the content of NH4$^+$-N in soil [36]. Therefore, the high concentration of NH4$^+$-N in the rhizosphere soil reduced

the pH, and some microbiomes could not adapt to the acidic environment, resulting in a decrease in diversity.

Bacteria are the most widely distributed and abundant microbial group in soil. As active biological factors in the rhizosphere ecological environment, rhizosphere bacteria can participate in various biochemical processes in soil [37], which is of great significance for improving soil fertility, regulating plant growth, and maintaining ecosystem stability. In this study, Actinobacteria, Proteobacteria, Acidobacteria, and Chloroflexi were the dominant bacteria in the rhizospheres of the three soil types; this finding was consistent with the results of related research on the identification of the dominant bacteria in the soil [38]. Actinobacteria are thought to be more abundant in forest and plantation soils [39], consistent with the results of this study. Members of Actinobacteria have the ability to turnover organic matter and degrade recalcitrant molecules [39]. Moreover, they have an important role in biological methods of plant protection [40]. Thus, all these soil functions including promoting growth, symbiosis, and elicitors of plant resistance, added to potential implications in nitrogen cycling [41], so that Actinobacteria can find appropriate ecological niches in the ecosystem and can be widely existed. In addition, the abundance of Firmicutes in the rhizosphere was greater than 10% in only the Cambisols, where the abundance of this phylum was much higher than that in the other soil types, which was related to the soil environment and the preferences of bacteria. Firmicutes are mostly involved in the decomposition of and nitrogen fixation from organic matter, and species aggregation is related to the soil pH, C/N ratio, clay content, and other indicators [42]. These results indicated that Firmicutes prefers loose soil with a high pH and rich organic matter content and that specific soil rhizosphere environments will attract specific bacterial aggregations.

4.2. Biomarkers of Rhizosphere Bacteria in the Different Soil Types of Pecan Plantations

Rhizosphere microbiome development is mainly driven by the secretion of relevant substances by plant roots into the soil; these substances induce the accumulation of microbiomes needed for growth in the rhizosphere and influence soil pH and mineral element morphology, thus changing the rhizosphere environment and creating an obvious rhizosphere effect [43]. In this study, at the phylum level, the species of rhizosphere bacteria in the different soil types of pecan plantations were similar, but their abundances were slightly different. This result showed that within the same plant variety, even in different soil environments, the community composition of rhizosphere bacteria recruited by root exudates during plant growth was consistent at relatively high classification levels. However, Prober et al., pointed out that soil environmental factors had a greater influence on microbiomes than plant factors at the local range [44]; indeed, when we refined the classification level, we found that there were great differences among the members of the rhizosphere bacterial community at the family level. LEfSe analysis showed that there were significantly enriched rhizosphere bacteria in the different soil types of pecan plantations, and these biomarkers were also important nodes in the ecological network, playing key roles in the rhizosphere environment. Microbiomes with rhizosphere enrichment effects can directly reflect soil nutrient transformation and microbial environmental adaptability [45]. Xanthobacteraceae is a type of bacteria that is efficient in nitrogen fixation [46], and has an important positive effect on plant growth. Although norank_o__Acidobacteriales has no clear classification name, it belongs to the Acidobacteria, which have been proven to have potential ecological functions, such as degrading plants [47] and participating in iron cycling [48]. Both norank_o_Acidobacteriales and Xanthobacteraceae can grow in acidic soils [49], and their adaptability to pH could allow them to become significantly enriched in the rhizosphere soils of Luvisols. Nocardioidaceae is a family of Actinomycetes that can produce a variety of antibiotics and play an important role in soil improvement [50]. This taxon is mostly concentrated in extreme environments such as saline and alkaline areas [51]. Therefore, it could be one of the rhizosphere biomarkers of Solonchaks. Bacillaceae is considered a safe bacteria for plants and shows strong activation activity in the production of indole acetic acid (IAA), hydrolase, and antibiotics [52]. In addition, Bacillaceae can

transfer insoluble nutrients to an absorbable state to improve the rhizosphere nutrient environment, especially in phosphate solution [53]. Therefore, enrichment of Bacillaceae in Cambisols rhizospheres may increase the contents of soil available nutrients to a certain extent. In this study, we also found a group of bacteria that were not highly abundant but still had high network parameters in the rhizosphere network and were important members of the core microbiome. For example, Bryobacteraceae has hydrolytic capabilities and can utilize various sugars and polysaccharides [54] and plays an important role in the process of increasing available carbon utilization efficiency [55]. This taxon also supports community recovery and improves the ability of community resistance disturbances [56]. Haliangiaceae represents a unique myxobacterial taxon. Although few studies of this taxon have been performed, Haliangiaceae has been proven to have high metabolic activity in the soil microbiome [57] and may have a potential impact on community composition. Consistent with the results of previous studies [58], this result showed that some species with lower relative abundances can still play important roles in rhizosphere soil through their functional characteristics and relatively strong community linkages.

4.3. Potential Functions of Rhizosphere Bacteria in the Different Soil Types of Pecan Plantations

The microbiome is generally regarded as the driver of host plant functions and the regulator of soil nutrient cycling. By enhancing genomic and metabolic capacities, the microbiome can promote plant nutrient absorption and improve biological resistance [59]. Moreover, a reciprocal system of element conversion can be established to improve the soil environment and soil health. Therefore, the functional composition can reflect the interaction, regulation, and adaptability of the rhizosphere microbiome at the plant–soil interface to a certain extent. PICRUSt is a commonly used method that predicts the potential functions of bacteria by comparing gene sequences with different databases [60]. The impacts of soil properties on bacterial diversity will further affect functional composition [61]. In this study, there were different numbers of genes assigned to the database in the different rhizosphere samples, and the Cambisols had the highest number of genes. The higher number of functional genes indicated that the rhizosphere environment in the part of pecan with Cambisols supported a higher diversity of bacteria and promoted the metabolic capacity of the bacterial community, which manifested as fast growth and excellent adaptability, thus indicating an advantage in functional range and abundance. The KO and pathway-level heatmaps demonstrated the diversity and richness of rhizosphere bacterial functions. These functional genes can promote amino acid absorption [62], improve nitrogen fixation and phosphorus solubilization [63], and participate in metabolite and ion transmembrane transport [64], and thus play positive roles in bacterial survival and reproduction, soil nutrient cycling, and plant growth and development. The functional profiles of the soil samples reflected the mutual benefits established between rhizosphere bacteria and pecan plantations at the plant-soil interface. In addition, in this study, the abundance of metabolism and genetic information processing functional layers was the highest in the Luvisols. The composition of rhizosphere bacteria in different soil types may explain this result. The research showed that Acidobacteria is a metabolically and genetically diverse group compared with the other phylum groups [65]. Thus, the high abundance of Acidobacteria in the Luvisols may result in a different abundance of corresponding functional layers in different soil types. However, due to the limitations of the PICRUSt functional prediction method, only potential functions were identified, so it is necessary to combine metagenomics and other analysis techniques to further uncover and verify the functions of rhizosphere bacteria.

5. Conclusions

Although the sources and compositions of rhizosphere microbiomes are complex, our study showed that the pecan plantations formed specific rhizosphere bacterial communities during the growth process and that these microbiomes established a beneficial growth system at the plant-soil interface, with functional characteristics such as plant growth

promotion and soil nutrient cycling. The accumulation of rhizosphere bacteria is obviously affected by the soil environment, which is influenced by factors such as soil type and soil property. In this study, pH was the primary environmental factor affecting rhizosphere bacteria, so the effects of pH on soil health, nutrient utilization, and the rhizosphere microbiome should be considered in the cultivation and management of pecan plantations. In addition, keystone species are not necessarily highly abundant in microbiomes. In the rhizosphere environments of pecan plantations, there existed a group of bacteria with low relative abundances that played important roles due to their functional characteristics and community association networks. Overall, this study provides a basis for the study of the rhizosphere microbiome in different soil types of pecan plantations, and plays an important role in the sustainable management of forest soil.

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