

ARTICLE

Planting Years Drive Structural and Functional Shifts in the Rhizosphere Bacterial Microbiome of *Zanthoxylum bungeanum*

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ABSTRACT: This study investigated the effects of planting duration (1, 5, 10 and 15 years) on soil properties, bacterial community diversity, and function in the rhizosphere of *Zanthoxylum bungeanum*. We employed Illumina high-throughput sequencing and PICRUSt2 functional prediction to analyze the structure and functional potential of rhizosphere soil bacterial communities. The Mantel test and redundancy analysis were used to identify physicochemical factors influencing bacterial community structure and function. The results indicated significant differences in rhizosphere soil physicochemical properties across planting years: the content of organic matter, alkaline hydrolyzable nitrogen in the soil, as well as the activity of invertase, urease, and alkaline phosphatase initially increased and then decreased, while available potassium, Olsen-phosphorus content, and peroxidase activity continued to increase. However, bacterial alpha diversity (Chao1 and Shannon indices) and the number of amplicon sequence variants increased continuously with planting duration. Principal coordinate analysis and Adonis tests revealed that the planting year significantly influenced the bacterial community structure ($p < 0.05$). The phyla *Proteobacteria*, *Actinobacteria*, *Acidobacteriota* and *Chloroflexi* collectively constituted 56.7% to 71.2% of the relative abundance, representing the dominant taxa. PICRUSt2 predictions indicated key functional categories (cellular processes, metabolism, genetic information processing, and environmental information processing) each exceeding 10% relative abundance. BugBase analysis revealed a progressive increase in aerobic and oxidative stress-tolerant bacteria and a decrease in anaerobic and potentially pathogenic bacteria. Differential indicator species analysis identified *Firmicutes*, *Planctomycetes*, *Methylomirabilota* and *Actinobacteriota* as key discriminators for the 1-, 5-, 10- and 15-year stages, respectively. Organic matter, alkaline phosphatase, soil pH, and available phosphorus were the primary physicochemical drivers of bacterial communities. Notably, soil organic matter significantly influenced both the community structure ($p < 0.05$) and predicted metabolic functions ($p < 0.05$). In conclusion, prolonged planting duration significantly enhanced rhizosphere microbial diversity and functional gene abundance in *Z. bungeanum* while driving the structural succession of bacterial communities dominated by *Proteobacteria*, *Actinobacteria*, *Acidobacteriota*, and *Chloroflexi*. This ecological shift, characterized by increased aerobic/oxidative-stress taxa and decreased anaerobic/pathogenic bacteria, was primarily regulated by soil organic matter, a key driver shaping both community structure and metabolic functions, ultimately improving soil microecological health.

KEYWORDS: *Zanthoxylum bungeanum*; planting years; rhizosphere soil; bacterial community; function prediction

1 Introduction

Soil microorganisms are integral components of ecosystems and play a pivotal role in maintaining forest ecosystem functions and soil nutrient cycling [1]. The diversity and composition of soil microbial



communities are highly sensitive to environmental changes [2], particularly to factors such as precipitation, temperature, soil pH, and nutrient availability [3]. Consequently, microbial community attributes are key indicators of soil quality and health. Vegetation significantly influences the diversity and composition of soil bacterial communities by altering the physicochemical properties of soil [4]. Furthermore, in healthy soils, a diverse and structurally stable microbial community ensures the proper functioning of its constituent functional groups, ultimately enhancing plant productivity [5].

The composition and diversity of soil microbial communities undergo dynamic shifts over time [3]. Previous studies have confirmed that forest planting duration significantly influences microbial community composition and diversity. As planting years increase, the abundance, community structure, and diversity of rhizosphere microorganisms undergo marked changes [6]. Studies have indicated that divergent responses among plant species and rhizobacterial diversity in *Swietenia macrophylla* increase with planting time [7], whereas in peonies, both the abundance and diversity of rhizosphere bacteria decline with longer planting duration [8]. Furthermore, soil characteristics such as nutrient status and enzyme activity drive changes in soil microbial community composition and diversity [9]. A study by Ye et al. [10] on *Torreya grandis* rhizosphere soil microorganisms revealed that although planting duration did not significantly affect bacterial diversity, it significantly altered microbial community composition. Furthermore, planting duration, C/N ratio, total soil nitrogen, and organic matter content were the key drivers shaping the rhizosphere microbial community structure. These findings underscore the pronounced plant species specificity in the response of rhizosphere bacterial communities to planting time. Therefore, the planting duration of *Z. bungeanum* may shape the composition and diversity of soil microbial communities by altering the rhizosphere soil properties. An in-depth analysis of the rhizosphere bacterial community composition and diversity across different planting ages will help elucidate the response mechanisms of soil bacteria to forest planting duration and environmental factors, which are crucial for promoting sustainable development in economic forest management.

The Linxia Hui Autonomous Prefecture in Gansu Province is a major production area for *Zanthoxylum bungeanum* in China. In recent years, the local *Z. bungeanum* cultivation area has expanded rapidly, and it has emerged as the primary economic forest crop in the prefecture. However, increasing planting duration (particularly under semi-natural cultivation) combined with extensive management practices has led to increasingly prominent issues of declining quality and productivity of *Z. bungeanum* in the region [11]. Extensive research has demonstrated that increasing tree-planting duration reduces soil microbial diversity and diminishes the abundance of key functional microbial groups. Consequently, these alterations lower soil productivity and increase disease incidence [12], severely compromising the ecological and economic benefits of trees. Currently, the mechanisms by which planting duration affects bacterial diversity and community composition in *Z. bungeanum* rhizosphere soil remain unclear.

Therefore, this study employed high-throughput sequencing to analyze the diversity and composition of rhizosphere soil microbial communities in *Z. bungeanum* across different planting durations. By integrating soil physicochemical properties and enzyme activity data, we explored the correlations between the microbial community structure and soil environmental factors. The results aimed to clarify the drivers of rhizosphere microbial community evolution and provide a scientific foundation for the sustainable management of *Z. bungeanum* industry in the Linxia Prefecture.

2 Overview of the Research Area

The research area is located in Lujia Village, Lianhua Town, Linxia County, Gansu Province (102°41'–103°40' E, 34°57'–36°12' N). Situated at an elevation of 1700 m, it experiences a typical continental monsoon climate characterized by distinct seasonal variations: dry winters and springs and humid summers and

autumns. The region receives an average annual rainfall of 385 mm, has a mean annual temperature of 6.8°C, and enjoys a frost-free period of approximately 170 days.

3 Materials and Methods

3.1 Sample Collection and Processing

Conduct field investigation and soil sample collection in Lujia Village, Lianhua Town, Linxia County, Linxia Prefecture, Gansu Province, China in June 2023, survey plots were established in artificially planted *Z. bungeanum* stands of different ages (1, 5, 10, and 15 years) using a spatial rather than a temporal approach. The site conditions were consistent across the designated plots (RS1, RS5, RS10 and RS15). Rhizosphere soil samples were collected within a 50 cm radius of each trunk from four cardinal directions (east, west, south, and north). Within each age class, the plants were grouped into three replicate sampling areas. Rhizosphere soil was collected as previously described [13]. Soil from the four directions around each sampled plant was combined into an equal composite sample. Subsequently, composites from the 3 replicate plants within each age group were pooled to form a single representative sample per age group, yielding a total of 12 samples. The samples were transported to the laboratory in insulated containers with ice packs. Large rocks and plant debris were removed with sterile tweezers. One portion of each sample was sieved (2 mm) and stored at −80°C for bacterial diversity analysis, while the remaining portion was air-dried and sieved (1 mm) for physicochemical property assessment.

3.2 Determination of Soil Physical and Chemical Properties

The chemical properties of the soil were assessed using the methods outlined in the agricultural soil analysis [14]. The organic matter content was measured using the potassium dichromate oxidation-ferrous sulfate titration method, alkali hydrolyzed nitrogen (AN) content was evaluated using the alkaline hydrolysis diffusion method, available phosphorus (AP) content was determined using the Olsen method, available potassium (AK) content was measured using the flame photometry method, and soil pH value was assessed using the potentiometric method.

3.3 Determination of Soil Enzyme Activity

Soil enzyme activities were determined according to the methods outlined in a previous study [15]. Peroxidase (POD) activity was assessed using the potassium permanganate titration method, while invertase (IE) and cellulase (CL) activities were evaluated using the 3,5-dinitrosalicylic acid colorimetric method. Urease (UE) activity was measured using the phenol-sodium hypochlorite colorimetric method and alkaline phosphatase (AKP) activity was determined using the disodium phenyl phosphate colorimetric method.

3.4 DNA Extraction and High-Throughput Amplicon Sequencing

Total soil DNA was extracted from 1.0 g of the frozen soil samples using a soil DNA extraction kit (MP Biomedicals, 116560200, Irvine, CA, USA). High throughput sequencing of rhizosphere soil bacteria using Illumina MiSeq platform from Lianchuang Biotechnology Co., Ltd. (Hang Zhou, China). The V3-V4 region of 16S rDNA, amplified with primers 341F (5'-CCTACGGGNGGCAGCAG-3') and 805R (5'-GACTACHVGGTATCTAATCC-3'), was subjected to sequencing analysis. Raw sequencing data were processed using the Quantitative Insights into Microbial Ecology2 (QIIME2, version 2019.4) software. Paired-end reads were assigned to the respective samples based on unique barcodes and were subsequently truncated by removing the barcodes and primer sequences. Amplicon sequence variants (ASVs) were generated by clustering high-quality sequences using the DADA2 pipeline in QIIME2.

3.5 Statistical Analyses

One-way analysis of variance (ANOVA) was conducted to compare the significance of soil chemical properties, bacterial community diversity, and taxonomic composition across different planting durations using Tukey's least significant difference test. Statistical significance was assessed at $p < 0.05$. The species composition of different groups and common unique species was assessed using Venn diagrams analyzed with the "VennDiagram" package in R version 3.6.1. Alpha diversity was measured by calculating the Shannon and Chao1 indices using QIIME2 software. Beta diversity differences based on Bray–Curtis distance were visualized and quantified using principal coordinate analysis and permutational multivariate ANOVA (PERMANOVA). The relative abundance of functional genes in the samples was compared with Kyoto Encyclopedia of Genes and Genomes (KEGG) database information using PICRUSt2 software in R version 4.2. The correlation between differences in bacterial community structure and functional gene differences was analyzed using Mantel tests from the vegan package in R software. Redundancy analysis (RDA) was performed using Canoco 5 software to investigate the key physicochemical factors affecting soil bacterial classes and functional genes, followed by an Envfit test for validation purposes. To further confirm the species that positively responded to planting years, a heatmap analysis was used to characterize the dominant strains under different planting durations/years/time periods. Mantel tests and Pearson correlation analyses were conducted using the linkET package in R version 4.0.2 to simultaneously examine the drivers influencing microbial community composition at both the phylum and class levels. Random forest algorithms were used to study the roles of these factors in microbial community assembly [16].

4 Results

4.1 Changes in Soil pH and Nutrient Content in *Z. bungeanum* Rhizosphere in Different Planting Years

A single-factor ANOVA was conducted to examine the pH and nutrient content of *Z. bungeanum* rhizosphere soil across different planting years, revealing significant differences in organic matter, AN, AP, and AK content among the planting years ($p < 0.05$). No significant differences ($p > 0.05$) were observed in the pH (Fig. 1B). According to Fig. 1A,C, there was a trend of initially increasing and then decreasing levels of organic matter and An with the increasing planting years. The peak values were reached at year 10, which were 1.88 times higher than those at year 1 for organic matter and 1.60 times higher than those at year 15 for AN. From Fig. 1D,E, it can be observed that the content of AP and AK showed a continuous upward trend, reaching its highest point at 15 years, which was significantly higher than that at the other stages.

4.2 Differences in Enzyme Activity in the Rhizosphere Soil of *Z. bungeanum* with Different Planting Years

A single-factor ANOVA was conducted to examine POD, IE, UE, AKP, and CL activities in the rhizosphere soil of *Z. bungeanum* plants grown for different years. The results showed significant differences ($p < 0.05$). Table 1 shows that POD activity increased continuously with planting time, reaching a maximum value at 15 years, which was 2.89 times higher than that at year 1. IE, UE, and AKP activities initially increased and then decreased, peaking at 10 years and significantly surpassing those at both 1 and 15 years by factors of 1.28, 1.45, and 1.10, respectively, compared with the activity levels observed at year 1; similarly, they were higher by factors of 1.24, 1.41, and 1.20, respectively, compared with the activity levels observed at year 15. The activity level of CL remained unchanged over the period from year 1 to 15 (Table 2).

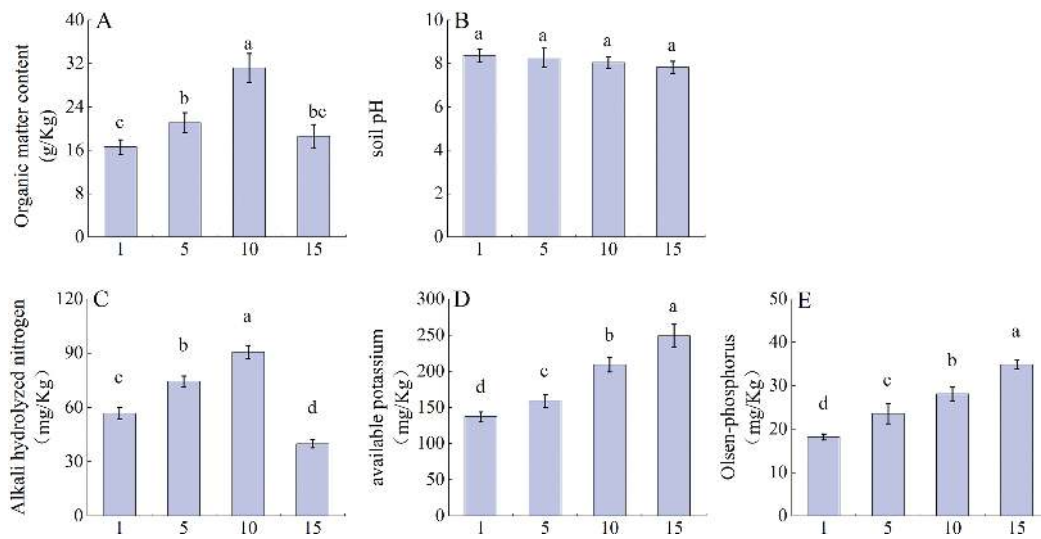


Figure 1: Changes in pH value and nutrient content of the rhizosphere soil of *Z. bungeanum* in different planting years. (A) organic matter content; (B) soil pH; (C) alkali hydrolyzed nitrogen content; (D) available potassium content; (E) Olsen-phosphorus content. Results are presented as mean values with standard errors ($n = 3$) $\pm$ SE. Different lowercase letters 'a–d' indicate significant differences ($p < 0.05$) between different planting years

Table 1: Detailed distribution of sampling sites in *Z. bungeanum* with different planting years

Planting years	Longitude (°E)	Latitude (°N)	Altitude (m)
1	103°19'03''	35°50'21''	1742
5	103°20'16''	35°51'36''	1743
10	103°19'35''	35°51'42''	1754
15	103°21'27''	35°50'14''	1746

Table 2: Changes in enzyme activities in the rhizosphere soil of *Z. bungeanum* in different planting years

Planting years	Peroxidase activity	Invertase activity	Urease activity	Alkaline phosphatase activity	Company: U/g Cellulase activity
1	0.09 $\pm$ 0.02b	35.36 $\pm$ 0.28c	2243.79 $\pm$ 20.52c	10,429.39 $\pm$ 76.32b	11.41 $\pm$ 0.05a
5	0.12 $\pm$ 0.02b	38.63 $\pm$ 0.38b	2680.16 $\pm$ 18.91b	11,255.13 $\pm$ 68.15a	12.33 $\pm$ 0.11a
10	0.24 $\pm$ 0.02a	45.09 $\pm$ 0.06a	3248.15 $\pm$ 17.35a	11,451.39 $\pm$ 58.63a	11.32 $\pm$ 0.10a
15	0.26 $\pm$ 0.01a	36.41 $\pm$ 0.02c	2301.89 $\pm$ 31.08c	95,79.81 $\pm$ 43.59c	11.81 $\pm$ 0.04a

Note: Results are presented as mean values ($n = 3$) $\pm$ standard error (one-way ANOVA, $p < 0.05$). Different lowercase letters 'a–c' indicate significant differences ($p < 0.05$) between different planting years.

4.3 Analysis of ASVs between Planting Years

Twelve soil samples were processed with a minimum sample sequence number of 44,412, resulting in the identification of 25,592 ASVs by clustering at a similarity threshold >97%. The sequencing coverage for each sample exceeded 99.72%, indicating that the obtained sequence information adequately represented the true status of soil bacteria in the study area.

The Venn diagram in Fig. 2 compares the bacterial ASVs detected in *Z. bungeanum* rhizosphere across different planting years. From Fig. 2B, it is evident that RS10 and RS15 exhibited significantly higher numbers of ASVs than RS5 and RS1. Among these samples, only 3.11% of ASVs were shared, suggesting substantial differences in the rhizosphere bacterial community composition between the different planting years. In pairwise comparisons among these four samples, the smaller was the difference in planting years, the higher was the consistency of the soil bacterial communities; conversely, as the difference in planting years increased, the consistency decreased. Notably, both RS15 and RS10 had significantly higher numbers of ASVs than RS5 and RS1. Specifically, compared with RS1, the number of ASVs in RS10 and RS15 increased by 2017 and 3061, respectively. The unique ASV counts in RS1, RS5, RS10, and RS15 were 1666, 1531, 3087, and 3477, respectively (Fig. 2A). RS15 had the highest unique bacterial group, whereas RS1 has the lowest, accounting for total ASV of 26.32% and 12.61%, respectively.

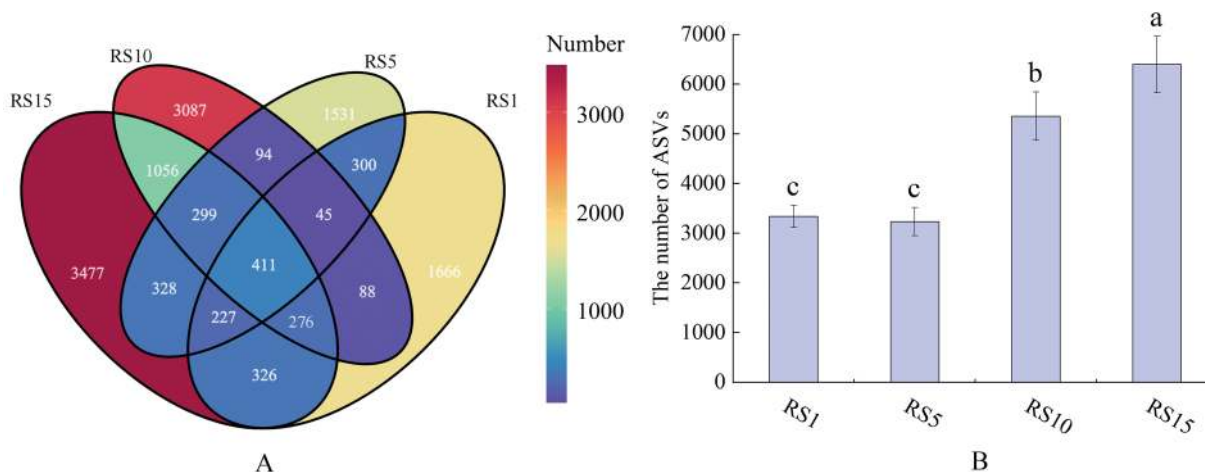


Figure 2: Venn diagram illustrating the bacterial composition in the rhizosphere soil of *Z. bungeanum* across different years of cultivation. (A) the number of ASVs shared and unique to each sample; (B) the number of ASVs for each sample. RS1: planting for 1 year, RS5: planting for 5 year, RS10: planting for 10 year, RS15: planting for 15 year. Different letters 'a–c' indicate significant differences in data between groups ($p < 0.05$)

4.4 Diversity Analysis of Rhizosphere Bacterial Communities under Different Planting Years

From Fig. 3A,B, it is evident that the Chao1 index (a richness index) in RS15 exhibited a significantly higher value compared with that in RS1 and RS5, with increases of 1.61 and 0.33 times, respectively. The Shannon index (diversity index) of RS15 and RS10 was significantly higher than that of RS1 and RS5 ($p < 0.05$). In terms of β -diversity, there was an increasing trend in bacterial community β -diversity with longer planting years (Fig. 3C). Principal coordinate analysis based on the Bray-Curtis distance algorithm revealed the spatial separation of rhizosphere soil bacterial communities in different planting years (excluding RS1 and RS5) (Fig. 3D). The principal component axes PC1 and PC2 explained 28.67% and 14.22% of the variation in the bacterial community structure, respectively. Furthermore, the Adonis test revealed significant differences in the rhizosphere bacterial community structure among *Z. bungeanum* plants with varying planting years

($R^2 = 0.475$, $p = 0.002$). As the duration of planting increased, the biological replicates became more closely clustered and the distances between different groups increased, indicating a significant impact of planting duration on rhizosphere bacterial communities.

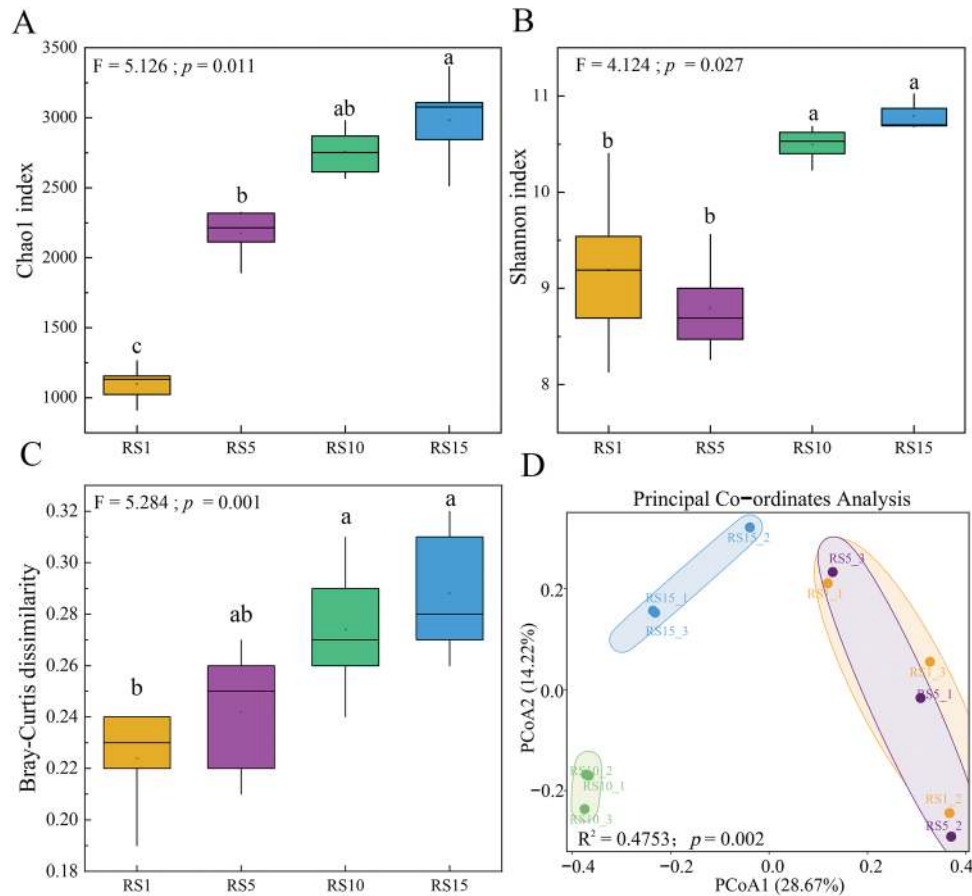


Figure 3: Diversity of bacterial communities in *Z. bungeanum* across different planting years. (A,B) Boxplot displayed the differences in the Chao1 index and Shannon index of bacterial communities under different planting years. (C) The pairwise Bray-Curtis dissimilarity of bacterial communities under different planting years. Different letters 'a–c' represent significant differences ($p < 0.05$) among groups according to the least significant difference (LSD) test. (D) Principal-coordinate analysis (PCoA) of bacterial communities based on Bray-Curtis distances, different colors represent different groups, with points of the same color indicating different samples within a group. The same group is displayed in a circular chart format according to the 95% confidence interval

4.5 Analysis of Soil Bacterial Community Structure in Rhizosphere of *Z. bungeanum* with Different Planting Years Based on Classification Status

The composition of the rhizosphere bacterial communities remained consistent across different planting years, but there were variations in the relative abundance. A total of 50 phyla, 150 classes, 326 orders, 531 families, and 1095 genera of bacteria were detected in all soil samples. Nine phyla of soil bacteria had a relative abundance $\geq 1\%$, while those with a relative abundance $< 1\%$ were grouped into others. The abundance of each phylum varied at different stages (Fig. 4A). *Proteobacteria* (from 28.57% to 21.07%), *Actinobacteria* (from 11.56% to 24.52%), *Acidobacteriota* (from 10.68% to 15.61%), and *Chloroflexi* (from 5.86% to 9.98%) accounted for 56.67%–71.18% of the total bacterial relative abundance and were the dominant soil bacterial species. The relative abundance of *Proteobacteria* was significantly higher in RS1 and RS5 than in RS10 and RS15, and

the relative abundance of *Actinobacteria* in RS15 was significantly higher than that in RS1, RS5, and RS10 by 2.12, 1.70, and 1.15 times, respectively. The relative abundance of *Acidobacteriota* was significantly higher in RS15 and RS10 than in RS1 and RS5, while the relative abundance of *Chloroflexi* was significantly lower in RS1 than in others ($p < 0.05$). Additionally, *Planctomycota*, *Gemmatimonadota*, *Firmicutes*, *Bacteroidota*, and *Myxococota* accounted for a total of 18.86%–35.18% of rhizosphere soil bacteria across RS1 to RS15, making them the main bacterial phyla present. Among these phyla, the relative abundance of *Gemmatimonadota* was significantly higher in RS15 and RS10 than in RS5 and RS1, whereas the abundance of *Firmicutes* and *Bacteroidota* in RS1 was significantly higher than that in RS10 and RS15 ($p < 0.05$).

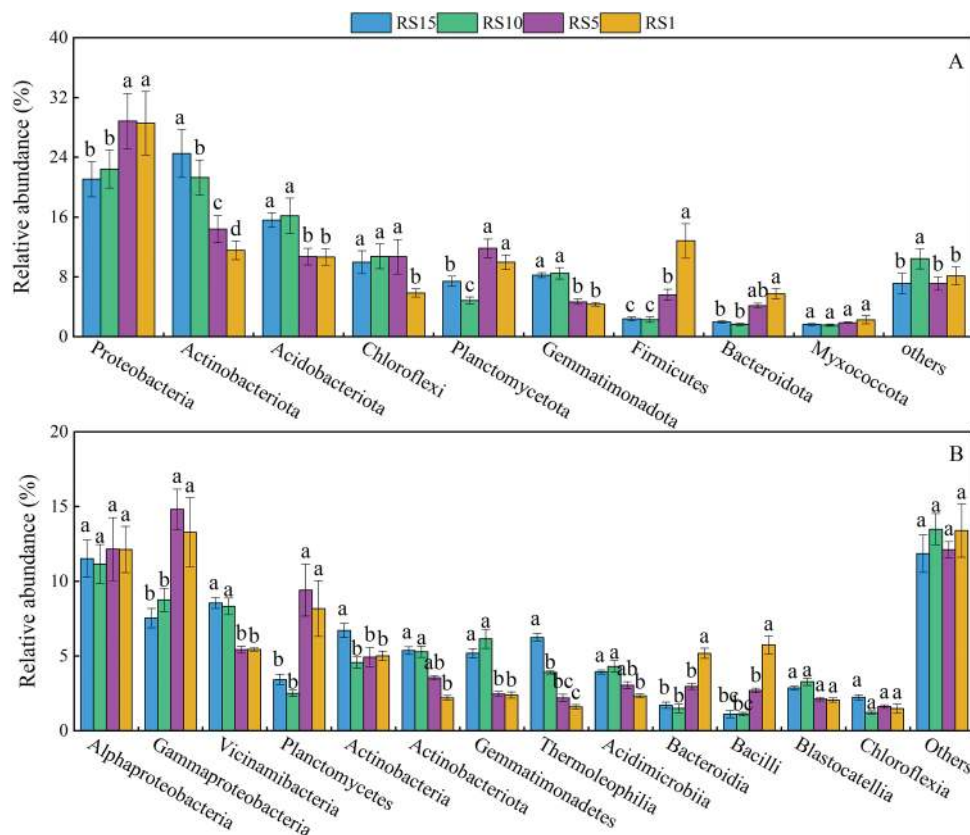


Figure 4: Composition of bacterial communities in *Z. bungeanum* across different planting years. (A) The relative abundance (%) of more than 1% dominant bacterial at the phylum level. (B) The relative abundance (%) of more than 1% dominant bacterial at the class level. Different letters ‘a–d’ represent significant differences ($p < 0.05$) among groups according to the least significant difference (LSD) test

Bacterial classes with an average relative abundance of $<1\%$ were combined into “Others,” while there were 13 classified bacterial classes with an abundance of $\geq 1\%$. The relative abundance of *Gammaproteobacteria* within the phylum *Proteobacteria* in RS1 increased significantly by 5.74% compared with that in RS15, *Planctomycetes* within the phylum *Planctomycetota* in RS1 increased significantly by 4.74% compared with that in RS15, *Bacteroidia* within the phylum *Bacteroidota* in RS1 increased significantly by 3.47% compared with that in RS15, and *Bacilli* within the phylum *Firmicutes* in RS1 increased significantly by 4.62% compared with that in RS15. In contrast, the relative abundance of *Actinobacteriota* within the phylum *Actinobacteriota* in RS1 decreased significantly by 3.16% relative to that in RS15, and *Gemmatimonadetes* within the phylum *Gemmatimonadota* in RS1 decreased significantly by 2.80% (Fig. 4B).

4.6 Effect of Bacterial Function in the Rhizosphere Soil of *Z. bungeanum* with Different Planting Years

Based on the 16S rRNA marker gene feature sequence, PICRUSt2 was used to predict the function of bacterial ASVs in the rhizosphere soil of different planting years of *Z. bungeanum*. The abundance of the KEGG Orthology for each sample was determined and statistically analyzed. The weighted NSTI evaluates the weighted average distance between the sequences in each sample and the genes measured in known databases. The smaller the NSTI value, the higher the degree of matching between the predicted microbial composition in the sample and the known database. The NSTI for predicting bacterial function in the rhizosphere soil of different tree ages is shown in Fig. 5. The NSTI of samples 1a, 5a, 10a, and 20a was 0.15–0.19, 0.16–0.20, 0.17–0.23, and 0.21–0.26, respectively (Fig. 5).

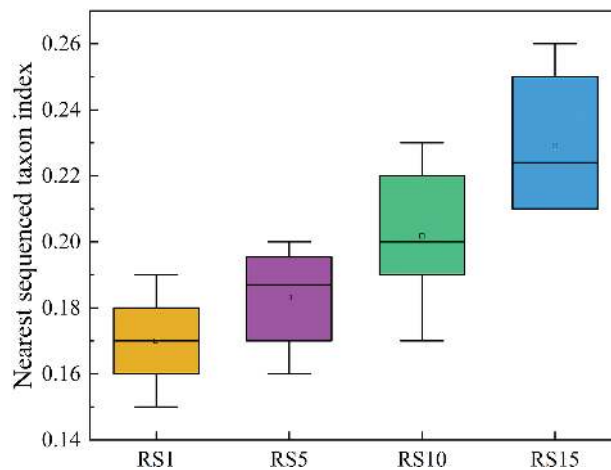


Figure 5: NSTI of picrust predictive functional profiling

A comparison with the KEGG database revealed seven categories of primary metabolic pathway functions, among which the functions with relative abundances >1% were as follows: metabolism (47.45%), genetic information processing (21.86%), environmental information processing (11.02%), and cellular processes (8.51%) (Table 3). In addition, the bacterial community distributed across seven metabolic pathways revealed 40 secondary functions, with 20 having a relative abundance >1%. Amino acid metabolism, carbohydrate metabolism, replication and repair, and membrane transport were identified as the main functional groups among the soil bacteria. The relative abundance of amino acid metabolism, carbohydrate metabolism, terpenoids, and polyketides metabolism in the rhizosphere soil bacteria of RS15 was significantly higher than that in the rhizosphere soil bacteria of RS1; however, cellular processes and signaling functions showed significantly lower abundance in RS15 than that in RS1 ($p < 0.05$). Furthermore, there were no significant differences in the relative abundance of other functions among the rhizosphere soil bacteria in the different planting years of *Z. bungeanum*.

Table 3: Relative abundance and differences in functional genes of soil bacteria in the rhizosphere of *Z. bungeanum* plants with different years of cultivation

Primary function	Functional	Relative abundance (%)			
	Secondary function	RS1	RS5	RS10	RS15
	Amino acid metabolism	10.07 ± 0.83 b	10.47 ± 0.05 ab	10.85 ± 0.02 ab	10.91 ± 0.03 a
	Carbohydrate metabolism	9.93 ± 0.12 b	9.91 ± 0.11 b	9.98 ± 0.02 ab	10.10 ± 0.03 a

(Continued)

Table 3 (continued)

Functional		Relative abundance (%)			
Metabolism	Energy metabolism	5.86 ± 0.29 b	6.03 ± 0.03 ab	6.17 ± 0.04 a	6.14 ± 0.08 ab
	Metabolism of cofactors and vitamins	4.16 ± 0.20 b	4.26 ± 0.01 ab	4.40 ± 0.03 a	4.36 ± 0.06 ab
	Nucleotide metabolism	3.91 ± 0.07 b	3.88 ± 0.03 b	4.02 ± 0.03 a	3.96 ± 0.07 ab
	Lipid metabolism	3.62 ± 0.42 a	3.83 ± 0.05 a	3.75 ± 0.01 a	3.85 ± 0.06 a
	Glycan biosynthesis and metabolism	2.27 ± 0.09 a	2.18 ± 0.07 a	2.27 ± 0.02 a	2.21 ± 0.06 a
	Xenobiotics biodegradation and metabolism	2.17 ± 0.26 a	2.32 ± 0.05 a	2.13 ± 0.05 a	2.33 ± 0.16 a
	Metabolism	2.01 ± 0.18 a	1.94 ± 0.05 ab	1.75 ± 0.02 c	1.80 ± 0.04 bc
	Metabolism of other amino acids	1.77 ± 0.09 a	1.79 ± 0.01 a	1.81 ± 0.01 a	1.84 ± 0.03 a
Genetic information processing	Metabolism of terpenoids and polyketides	1.67 ± 0.17 b	1.77 ± 0.00 ab	1.83 ± 0.01 ab	1.85 ± 0.01 a
	Replication and repair	8.41 ± 0.19 ab	8.35 ± 0.04 b	8.65 ± 0.07 a	8.48 ± 0.19 ab
	Translation	5.52 ± 0.09 b	5.56 ± 0.04 b	5.98 ± 0.09 a	5.75 ± 0.24 ab
	Folding, sorting and degradation	2.88 ± 0.11 a	2.93 ± 0.02 a	3.00 ± 0.02 a	2.94 ± 0.07 a
	Genetic information processing	2.59 ± 0.21 a	2.51 ± 0.01 a	2.46 ± 0.01 a	2.42 ± 0.04 a
Environmental information processing	Transcription	2.45 ± 0.19 a	2.38 ± 0.02 a	2.35 ± 0.00 a	2.35 ± 0.02 a
	Membrane transport	11.02 ± 0.95 a	10.57 ± 0.14 a	10.42 ± 0.14 a	10.32 ± 0.15 a
Cellular processes	Cellular processes and signaling	3.49 ± 0.45 a	3.25 ± 0.04 ab	2.93 ± 0.03 b	2.93 ± 0.07 b
	Cell motility	2.91 ± 0.15 ab	2.93 ± 0.07 a	2.73 ± 0.01 b	2.75 ± 0.11 ab
	Signal transduction	2.10 ± 0.09 a	2.07 ± 0.03 a	1.87 ± 0.01 b	1.82 ± 0.06 b

Note: Different letters 'a–c' represent significant differences ($p < 0.05$) among groups according to the least significant difference (LSD) test.

Pearson's correlation analysis indicated (Table 4) that there was a significant correlation between the relative abundance changes of secondary functional genes in the rhizosphere soil bacteria of *Z. bungeanum* and those of phyla *Acidobacteriota*, *Actinobacteota*, *Bacteroidota*, *Chloroflexi*, *Firmicutes* and *Gemmatimonadota*. This suggests that these bacterial groups may have a crucial effect on the function of rhizosphere soil bacteria associated with *Z. bungeanum*.

Table 4: The relationship between the relative abundance of secondary functions of soil bacteria in the rhizosphere of *Z. bungeanum* and the relative abundance of major bacterial phyla

Functional	Bacterial phylum								
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Amino acid metabolism	0.885**	0.850**	−0.987**	0.723**	−0.973**	0.836**	−0.061	0.044	−0.555
Carbohydrate metabolism	0.230	0.492	−0.025	−0.197	0.105	0.416	−0.194	−0.422	−0.807**
Cell motility	−0.400	−0.570	0.263	−0.251	0.119	−0.395	0.442	0.766**	0.623*
Cellular processes and signaling	−0.898**	−0.894**	0.974**	−0.735**	0.933**	−0.861**	0.167	0.096	0.641*
Energy metabolism	0.887**	0.788**	−0.969**	0.800**	−0.950**	0.761**	−0.153	−0.009	−0.534
Folding, sorting and degradation	0.861**	0.579	−0.868**	0.654*	−0.832**	0.651*	−0.008	0.030	−0.503

(Continued)

Table 4 (continued)

Functional	Bacterial phylum								
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Genetic information processing	−0.830**	−0.781**	0.947**	−0.616*	0.959**	−0.806**	−0.094	−0.216	0.476
Glycan biosynthesis and metabolism	0.056	−0.209	0.277	−0.511	0.434	−0.028	0.087	−0.355	−0.357
Lipid metabolism	0.693*	0.525	−0.838**	0.574	−0.913**	0.589	0.265	0.534	−0.245
Membrane transport	−0.857**	−0.702*	0.951**	−0.666*	0.964**	−0.744**	−0.091	−0.269	0.478
Metabolism	−0.913**	−0.876**	0.944**	−0.694*	0.873**	−0.872**	0.234	0.204	0.681*
Metabolism of cofactors and vitamins	0.916**	0.810**	−0.977**	0.743**	−0.937**	0.814**	−0.109	−0.069	−0.591
Metabolism of other amino acids	0.712**	0.740**	−0.861**	0.490	−0.883**	0.782**	0.178	0.243	−0.394
Metabolism of terpenoids and polyketides	0.856**	0.846**	−0.981**	0.760**	−0.975**	0.811**	−0.092	0.043	−0.527
Nucleotide metabolism	0.356	0.428	−0.149	0.067	0.038	0.401	−0.483	−0.787**	−0.692*
Replication and repair	0.190	0.296	−0.029	0.125	0.140	0.203	−0.607*	−0.864**	−0.474
Signal transduction	−0.866**	−0.892**	0.830**	−0.598	0.713**	−0.858**	0.342	0.446	0.795**
Transcription	−0.804**	−0.654	0.943**	−0.624*	0.974**	−0.748**	−0.190	−0.327	0.370
Translation	0.688*	0.654*	−0.600*	0.514	−0.460	0.618*	−0.456	−0.598	−0.686*
Xenobiotics biodegradation and metabolism	0.198	0.303	−0.455	0.360	−0.591	0.260	0.265	0.575	0.136

Note: P1, *Acidobacteriota*; P2, *Actinobacteriota*; P3, *Bacteroidota*; P4, *Chloroflexi*; P5, *Firmicutes*; P6, *Gemmatimonadota*; P7, *Myxococcota*; P8, *Planctomycetota*; P9, *Proteobacteria*. * $p < 0.05$, ** $p < 0.01$.

4.7 Bug BASE Bacterial Phenotype Prediction Analysis

The predicted bacterial phenotype of BugBase indicated that aerobic bacteria exhibited an upward trend with increasing planting years. The abundance of aerobic bacteria in RS15 was 1.5 times higher than that in RS1. In contrast, anaerobic bacteria displayed a downward trend, with *Acidobacteria* playing a dominant role owing to the differences in *Planctomycetes* abundance. Pathogenic potential bacteria showed a decreasing trend with increasing planting years; their abundance in RS15 was significantly lower than that in RS1, accounting for only 71.43% of the total abundance. This decrease was primarily driven by *Proteobacteria* and *Acidobacteria*. Bacterial resistance to oxidative stress was greater in 10a and 15a than in 1a and 5a; specifically, the abundance of stress-tolerant bacteria in RS15 was 1.3 times that in RS1 (Fig. 6).

4.8 Influence of Physicochemical Properties of the Rhizosphere Soil of *Z. bungeanum* with Different Planting Years on the Bacterial Community Structure and Metabolic Function

4.8.1 Influence of Physicochemical Properties of the Rhizosphere Soil of *Z. bungeanum* with Different Planting Years on Major Bacterial Phyla

To further explore the potential patterns of bacterial changes over the years of *Z. bungeanum* cultivation, indicator species analysis (Fig. 7A) revealed that *Firmicutes* was the differential indicator species at year 1, *Planctomycetota* at 5 years, *Methylomirabilota* at 10 years, and *Actinobacteriota* at 15 years. The relative abundance chart (Fig. 7B) also confirmed that as the planting years increased, the relative abundance of *Firmicutes* was the highest at year 1, *Planctomycetes* was particularly prominent at 5 years, and the relative abundance of *Methylomirabilota* and *Actinobacteriota* was higher at 10 and 15 years compared with other years.

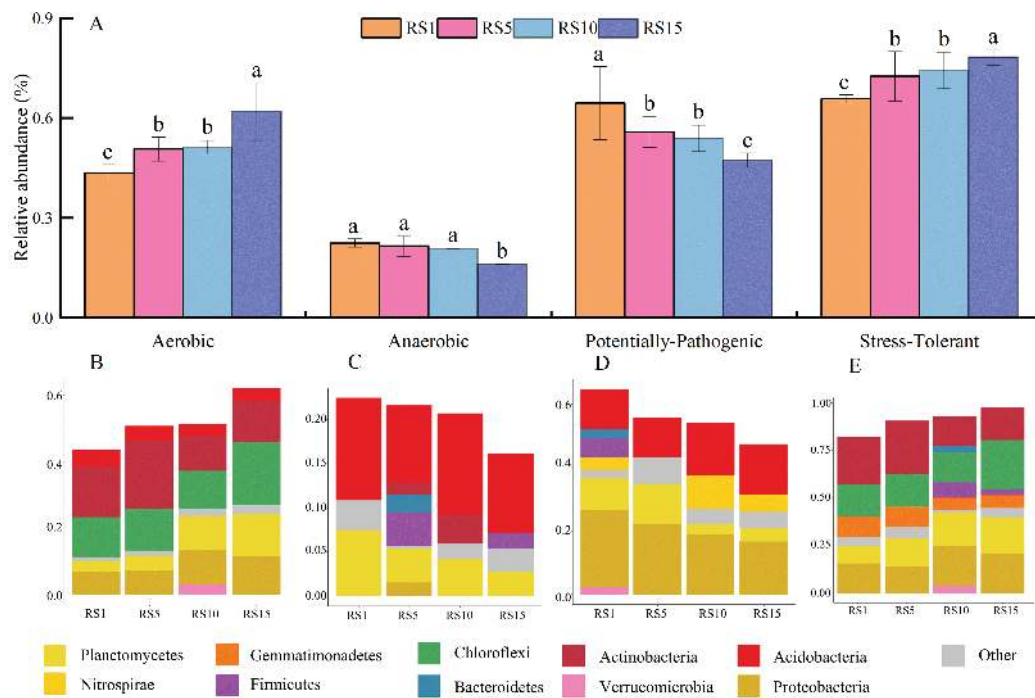


Figure 6: Phenotypic relative abundance (A) and composition of aerobic (B), anaerobic (C), potentially pathogenic (D), and stress-tolerant (E) bacteria in rhizosphere soil of different planting years. Different letters 'a-c' represent significant differences ($p < 0.05$) among groups according to the least significant difference (LSD) test

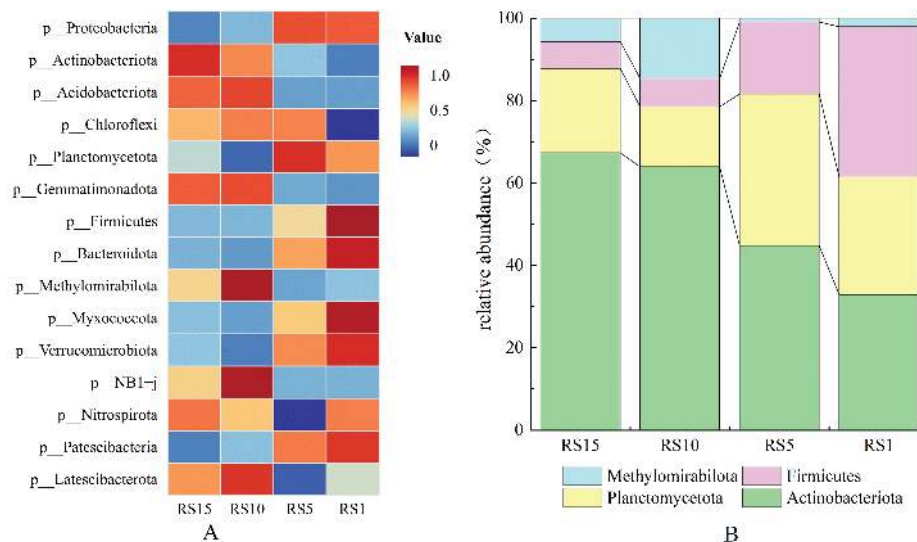


Figure 7: Differences in bacterial phylum levels in the rhizosphere soil of different planting years of *Z. bungeanum* indicate heatmap analysis (A) and relative abundance chart (B)

The main physicochemical factors affecting the indicator species of soil bacteria in the rhizosphere soil of *Z. bungeanum* with different planting years were explored using Mantel test correlation analysis. This analysis used the relative abundance distance matrix (Bray-Curtis) of indicator species from different planting years in the study area, as well as the Euclidean distance matrix of soil physicochemical parameters.

The results indicated that the indicator species *Firmicutes* (RS1) was correlated with AP and AK ($p > 0.05$); *Planctomycetota* (RS5) was correlated with POD ($p > 0.05$); *Methylobacteriota* (RS10) was significantly correlated with soil organic matter (SOM), AN, pH, POD, IE, and UE; and *Actinobacteriota* (RS15) was significantly correlated with AP, AK, pH, and POD ($p < 0.05$) (Fig. 8). Random forest analysis showed that SOM, AKP, pH, and AP were key predictive factors for bacterial community assembly in the rhizosphere soil of *Z. bungeanum* in different planting years (Fig. 9). As shown in Table 5, there is a highly significant positive correlation between *Methylobacteriota* and SOM; *Actinobacteriota* was significantly positively correlated with pH and AP ($p < 0.01$).

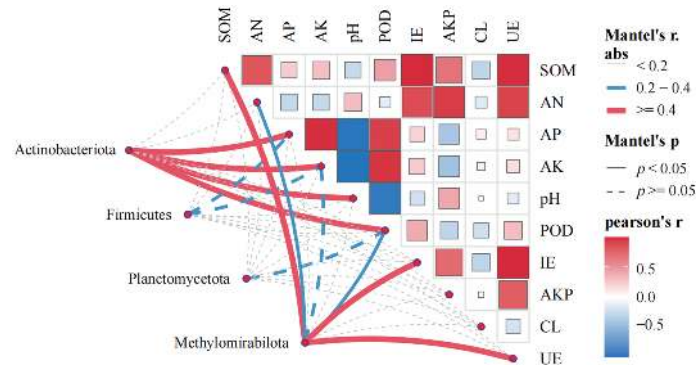


Figure 8: Correlation analysis between abiotic and biotic factors and the microbial community. Pairwise comparisons between factors are shown in a color gradient. Mantel tests for the correlations between factors and microbial community composition at the dominant phylum levels (Pearson's correlations, permutations = 9999). Abbreviations: SOM, soil organic carbon; AKP, alkaline phosphatase; pH, soil pH value; AP, available phosphorus; IE, Invertase; UE, urease; AN, available nitrogen; AK, available potassium; CL, cellulase; POD, peroxidase

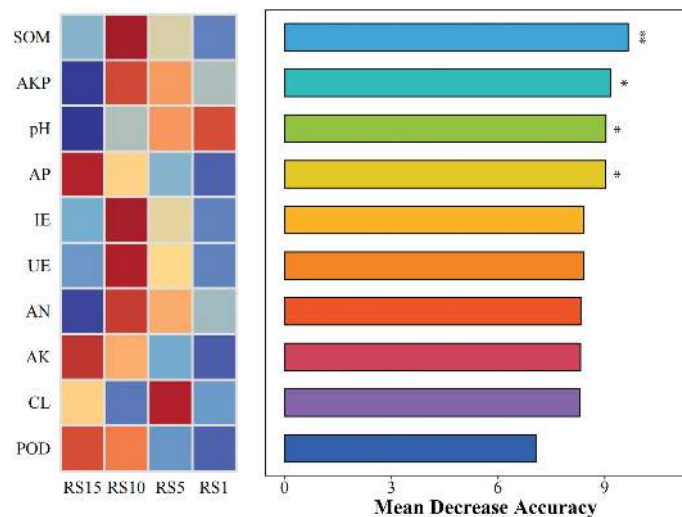


Figure 9: Random forests determined the role of plant diversity and soil properties in the bacterial communities' assembly. Abbreviations: SOM, soil organic carbon; AKP, alkaline phosphatase; pH, soil pH value; AP, available phosphorus; IE, Invertase; UE, urease; AN, available nitrogen; AK, available potassium; CL, cellulase; POD, peroxidase. Asterisks indicate significant differences (* $p < 0.05$, ** $p < 0.01$)

Table 5: Mantel significance test for differences in indicator species and major physicochemical factors of rhizosphere bacteria in different planting years

Planting years	Differential indicator species	Physical and chemical factors							
		SOM		AKP		pH		AP	
		r	p	r	p	r	p	r	p
1	<i>Firmicutes</i>	−0.044	0.322	−0.095	0.371	0.097	0.265	0.221	0.068
5	<i>Planctomycetota</i>	0.104	0.181	−0.168	0.949	0.051	0.277	0.058	0.260
10	<i>Methylobacteriota</i>	0.733	**	0.033	0.346	0.172	0.081	0.097	0.206
15	<i>Actinobacteriota</i>	0.035	0.370	0.167	0.134	0.775	**	0.798	**

Note: ** $p < 0.01$.

4.8.2 Influence of Physicochemical Properties of the Rhizosphere Soil of *Z. bungeanum* with Different Planting Years on Major Bacterial Classes

To further investigate the main environmental factors that affect the structure of soil bacterial communities in the rhizosphere soil of *Z. bungeanum*, detrended correspondence analysis was conducted on soil bacterial classes with a relative abundance of $\geq 1\%$ in the study area. Because the maximum value of the ordination axis was 2.1, RDA was performed using the relative abundances of the main soil bacterial classes as response variables and soil physicochemical indicators as the explanatory variables. The results indicated that the first and second sorting axes have explanatory powers of 55.15% and 21.11%, respectively, mainly determined by the first sorting axis, and the correlation coefficients between the first and second axes and soil physicochemical factors were 0.512 and 0.546, respectively, with a cumulative explanatory power of 94.55%, indicating that these two axes can better reflect the relationship between soil bacterial community structure and physicochemical factors (Table 6). Fig. 10 shows that *Vicinamibacteria*, *Actinobacteriota*, *Gemmatimonadetes*, *Thermoleophilia*, *Acidimicrobiia*, and *Blastocatellia* were positively correlated with SOM and AP, but negatively correlated with other classes. The Envfit test showed that both SOM and AP significantly affected the community structure of the bacterial class ($p < 0.01$), with contribution rates of 74% and 71%, respectively.

Table 6: Characteristic values of RDA ranking of soil bacterial classes and their explanatory power

Project	Rhizosphere bacterial community and physicochemical factors				Metabolic function and physicochemical factors			
	Axis 1	Axis 2	Axis 3	Axis 4	Axis 1	Axis 2	Axis 3	Axis 4
Characteristic values of relative abundance of rhizosphere soil bacteria	0.5515	0.2111	0.1083	0.0665	0.5995	0.2893	0.0464	0.0244
Cumulative explained amount of rhizosphere soil bacteria (%)	55.15	76.26	87.09	93.74	59.95	88.88	93.52	95.96
Correlation between rhizosphere soil bacteria and environmental factors	0.512	0.546	0.368	0.511	0.426	0.512	0.587	0.664
Cumulative explanatory power of rhizosphere bacteria and environmental factors (%)	87.26	94.44	97.65	99.47	89.65	95.63	98.11	99.59
Canonical eigenvalue			93.74				95.96	
Total eigenvalue			99.47				99.59	

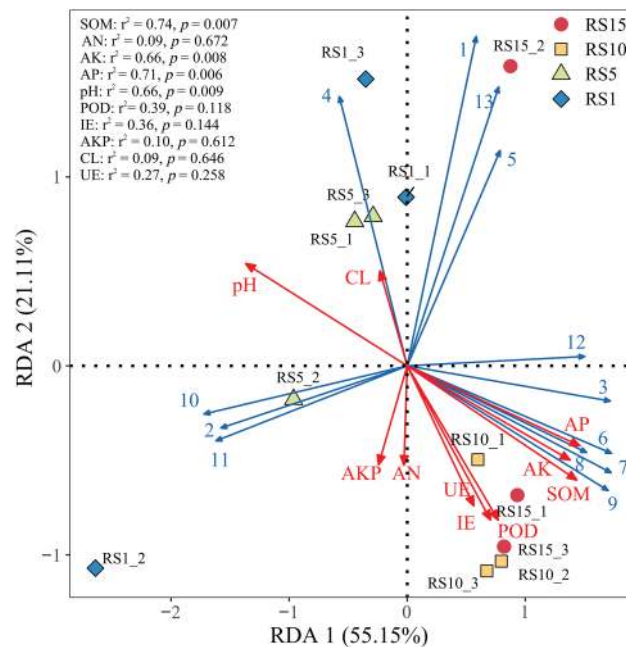


Figure 10: Redundancy analysis (RDA) of soil bacterial class level and physicochemical factors. Each point represents a sample, and the closer the distance between two points, the higher the similarity in community structure between the two samples. Arrows denote different influencing factors. An acute angle between the influencing factors (factors and samples) indicates a positive correlation, while an obtuse angle indicates a negative correlation. The longer the ray, the greater the effect of the factor. The position of the projection point on the arrow of the sample approximately represents the numerical magnitude of the factor in the corresponding sample. RS1, RS1: planting for 1 year, RS5: planting for 5 year, RS10: planting for 10 year, RS15: planting for 15 year. Abbreviations: SOM, soil organic carbon; AKP, alkaline phosphatase; pH, soil pH value; AP, available phosphorus; IE, Invertase; UE, urease; AN, available nitrogen; AK, available potassium; CL, cellulase; POD, peroxidase. Blue solid line: soil dominance class. 1, *Alphaproteobacteria*; 2, *Gammaproteobacteria*; 3, *Vicinamibacteria*; 4, *Planctomycetes*; 5, *Actinobacteria*; 6, *Actinobacteriota*; 7, *Gemmatimonadetes*; 8, *Thermoleophila*; 9, *Acidimicrobiia*; 10, *Bacteroidia*; 11, *Bacilli*; 12, *Blastocatellia*; 13, *Chloroflexi*

4.8.3 Effect of Physicochemical Properties of the Rhizosphere Soil of *Z. bungeanum* with Different Planting Years on the Relative Abundance of Metabolic Functions

RDA indicated that the metabolism of amino acids and carbohydrates in soil bacteria was positively correlated with the physicochemical properties of the rhizosphere soil (excluding pH and CL), while the replication and repair functions in genetic information processing were positively correlated with soil physicochemical properties (excluding pH and CL). Membrane transport in environmental information processing was negatively correlated with the physicochemical factors of soil (excluding pH and AKP) (Fig. 11). The Envfit test showed that soil organic matter had a significant effect on community bacterial function in the rhizosphere soil ($p < 0.01$), with a contribution rate of up to 64%.

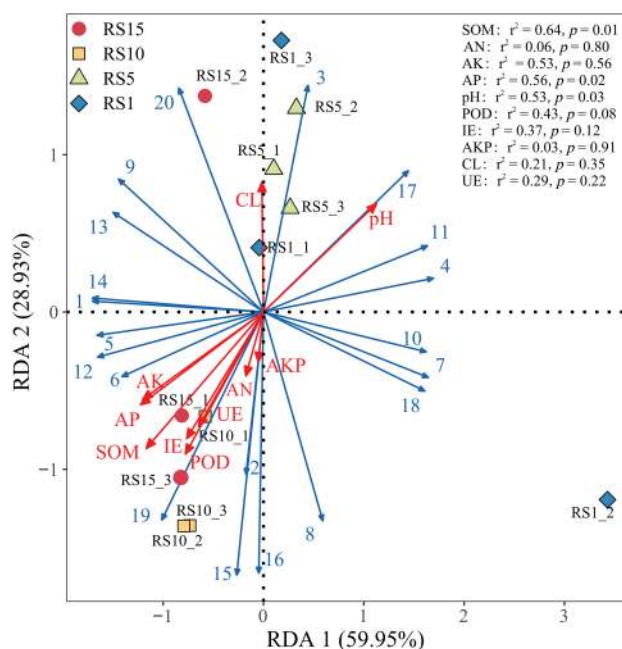


Figure 11: Redundancy analysis (RDA) of soil bacterial metabolic functional genes and physicochemical factors. Each point represents a sample, and the closer the distance between two points, the higher the similarity in community structure between the two samples. Arrows denote different influencing factors. An acute angle between the influencing factors (factors and samples) indicates a positive correlation, while an obtuse angle indicates a negative correlation. The longer the ray, the greater the effect of the factor. The position of the projection point on the arrow of the sample approximately represents the numerical magnitude of the factor in the corresponding sample. RS1, RS1: planting for 1 year, RS5: planting for 5 year, RS10: planting for 10 year, RS15: planting for 15 year. Abbreviations: SOM, soil organic carbon; AKP, alkaline phosphatase; pH, soil pH value; AP, available phosphorus; IE, Invertase; UE, urease; AN, available nitrogen; AK, available potassium; CL, cellulase; POD, peroxidase. Blue solid line: bacterial function in rhizosphere soil. 1, Amino Acid Metabolism; 2, Carbohydrate Metabolism; 3, Cell Motility; 4, Cellular Processes and Signaling; 5, Energy Metabolism; 6, Folding, Sorting and Degradation; 7, Genetic Information Processing; 8, Glycan Biosynthesis and Metabolism; 9, Lipid Metabolism; 10, Membrane Transport; 11, Metabolism; 12, Metabolism of Cofactors and Vitamins; 13, Metabolism of Other Amino Acids; 14, Metabolism of Terpenoids and Polyketides; 15, Nucleotide Metabolism; 16, Replication and Repair; 17, Signal Transduction; 18, Transcription; 19, Translation; 20, Xenobiotics Biodegradation and Metabolism

5 Discussion

5.1 Effect of Planting Years of *Z. bungeanum* on the Physicochemical Properties of Rhizosphere Soil

Plants obtain essential growth materials from the soil through their root systems, while simultaneously releasing photosynthetic byproducts into the surrounding soil as root exudates or decomposed roots. The composition and concentration of these substances fluctuate in response to external environmental changes, thereby influencing plant growth and development [17]. This study observed an initial increase followed by a decrease in organic matter and AN content in the rhizosphere soil of *Z. bungeanum* with increasing planting years, which is consistent with the findings reported by Jiao et al. [18], but this result differs from that reported by Liu et al. [19]. These discrepancies may be attributed to variations in *Z. bungeanum* management practices, including planting density [20], intercropping patterns [21], and the application of organic fertilizers, all of which influence the physicochemical properties of rhizosphere soil. Additionally, this may be associated with the vigorous growth phase of plants during this period, resulting in increased root activity and reduced nutrient accumulation. Some studies have found that long-term continuous cultivation

may lead to a decrease in soil nutrient availability [10] but may also result in cumulative benefits in terms of soil nutrient increase. For instance, Wang et al. [22] discovered significantly higher levels of AP and AK in the rhizosphere soil of *Rehmannia glutinosa* under continuous cropping than in the preceding crop and wild soils. This finding is in agreement with the findings of the present study, which may be due to the fact that acids, phytase and other substances secreted by *Z. bungeanum* root system play a key role in nutrient transformation process. Acids can lower the pH of rhizosphere soil, thereby increasing the solubility of certain nutrients. Phytase can break down phytic acid into inositol and phosphoric acid, releasing phosphorus that can be absorbed and utilized by plant, and these processes help to increase the amount of effective phosphorus and potassium in soil, which promotes the growth of *Z. bungeanum* [13]. The pH measurement results also showed that with the increase of planting years, pH value changed from weakly alkaline to weakly acidic, which is consistent with the findings of Liao et al. [23] and Jiao et al. [18]. In addition, correlation analysis also shows that pH value of rhizosphere soil is significantly negatively correlated with AP and AK content, which jointly supports the possibility of this mechanism.

Soil enzymes primarily originate from soil microorganisms, and the cultivation of crops over time can influence the quantity and diversity of these microorganisms, resulting in changes in soil enzyme activity. Soil enzyme activity can indirectly reflect or predict the transformation of certain nutrients and evolution of soil fertility [24]. In present study, POD activity in rhizosphere soil of *Z. bungeanum* increased continuously with years of planting, which may reflect the increased adaptation of *Z. bungeanum* to soil environment as well as its ability to detoxify harmful substances. This may reduce the accumulation of harmful substances, such as phenolic acids formed after plant decomposition, thereby lowering the risk of toxic effects on rhizosphere soil microorganisms [17]. The activities of IE, UE, and AKP are closely linked to organic matter transformation, nitrogen supply capacity, and organic phosphorus decomposition, respectively. In this study, correlation analysis revealed that IE activity has a highly significant or significant positive correlation with SOM content, IE with AN content, and AKP with AP content. Interestingly, the activities of IE, UE and AKP significantly decreased after 10 years, corresponding with changes in SOM and AN, which is consistent with the findings of Liu et al. [19]. This indicates that soil nutrient cycling is inhibited, which may lead to the slowing down of decomposition rate of SOM, insufficient supply of nitrogen and reduced effectiveness of phosphorus, thus affecting the growth of plants, which may be related to the changes in structure of soil microbial communities caused by long-term continuous cropping [25].

CL is a crucial indicator of carbon cycling rates in the soil, and its activity is influenced by nitrogen and phosphorus levels. Research indicated that CL activity increases with the age of walnut forests [26], whereas in apple orchards, it initially increases before declining [27]. However, we found no significant change in CL activity with increasing in years of *Z. bungeanum* cultivation. This suggests that CL activity responds differently to various factors, such as plant type, soil nutrients, microorganisms, vegetation, fertilization, and cultivation methods; thus, further investigation is necessary.

5.2 Effect of Planting Years on Bacterial Diversity and Community Structure in Rhizosphere Soil

The diversity and community structure of soil bacteria in the rhizosphere are highly complex and are influenced by factors such as fertilization management practices, forest age, soil type, plant species and planting duration. These factors result in dynamic changes in the bacterial population structure [18,28]. This study found that the planting years significantly affected the alpha diversity of bacteria in the rhizosphere soil of *Z. bungeanum*, which increased with the increase of planting years. These findings align with those of Wei et al. [29] regarding the relationship between different breeding years of grape plantations and the soil microbial community structure. The diversity trend observed in the soil microbial community structure gradually increased with the extended breeding years of Chinese fir plantations. This may be related to

the presence of root exudates in plants. *Z. bungeanum* is a typical allelopathic plant, and as the years of cultivation increase, the allelochemicals in its roots change, which may induce, attract, promote, or inhibit microorganisms, thereby affecting microbial diversity [30].

Proteobacteria, *Actinobacteria* and *Acidobacteria* were the dominant bacterial phyla (Fig. 4A), which is consistent with other forest soils [31,32]. In the present study, there were significantly different bacterial community compositions in different planting years (Fig. 4A,B). Different species appear in rhizosphere soil in different planting years owing to plants rhizosphere selectivity and varying preferences for bacteria at different stages. *Firmicutes* were identified as differential indicator species in the rhizosphere soil of 1-year-old *Z. bungeanum*, which may be related to the relatively high levels of organic matter and other nutrients in the rhizosphere soil of *Z. bungeanum* during its early developmental stages, which attract species such as fermenting and nitrogen-fixing bacteria [33]. This is consistent with the mechanism of reciprocal plant-microbe selection, in which the developmental stage of plants influences the assembly of microbial communities through nutrient-driven reciprocal interactions [34]. *Planctomycetes* have a unique cellular structure and lifestyle and play an important role in nutrient cycling [35]. In the rhizosphere soil of 5-year-old *Z. bungeanum*, we observed a shift in the differential indicator species to *Planctomycetes*. This may be due to the increase in planting years and photosynthetic products secreted by root system. This affects the propensity of different species to utilize the resource, leading to oxygenic photosynthesis and consequently the enrichment of a phylum of floxigenic molds that can survive in anoxic environments that utilize nitrite and oxidized ammonium ions [36]. This mechanism highlights selective screening and resource-driven microbial enrichment in the roots [37]. *Methylobacterium* was a differential indicator species for 10a rhizosphere soils, and correlation analyses showed that its abundance was significantly correlated ($p < 0.05$) with the alkaline dissolved nitrogen content, consistent with the results of He et al. [38]. This may be related to the over-application of nitrogen or nitrate fertilizers during the 10a period, which provided favorable conditions for its reproduction [39]. Overapplication of nitrogen may have increased competition for resources in favor of microorganisms adapted to high-nitrogen environments, suggesting reciprocal selection between the root system and bacteria mediated by fertilization practices [40]. *Actinobacteria* decompose SOM, promote carbon cycling, and support plant growth [41]. In the present study, the relative abundance of *Actinobacteria* gradually increased with the increasing years of planting and served as a differential indicator species in the 15-year old rhizosphere soil. This may be due to the continuous accumulation of complex compounds such as cellulose and lignin as well as root secretions in the soil, which provide more nutrient resources for the growth and reproduction of *Actinobacteria*, which is consistent with the findings of Cui et al. [42]. This optimizes soil nutrient cycling and microbial stability to maintain sustained plant growth [43]. This analysis suggests that plant-microbe reciprocal selection and inter-root selective screening play key roles in shaping bacterial community structure and function, and that factors such as cropping year, nutrient availability and plant physiology drive these dynamic processes [44]. Future research should focus on elucidating the molecular mechanisms underlying these interactions and explore strategies for utilizing microbial communities in sustainable agricultural practices.

5.3 Prediction of Bacterial Functional Genes in the Rhizosphere Soil of *Z. bungeanum*

Soil microorganisms play a crucial role in the decomposition of organic matter and the transformation of soil nutrients through their metabolic activities [45]. Metabolism is the primary function of soil bacterial communities during biogeochemical cycles [46]. In this study, the relative abundance of metabolic functions of soil bacteria in the rhizosphere soil of *Z. bungeanum* exceeded 48%, which was closely related to key functions such as carbohydrate metabolism, nitrogen fixation, and phosphorus solubilization. These functions promote nitrogen and phosphorus absorption by *Z. bungeanum* roots. Amino acid metabolism

facilitates nitrogen cycling through deamination and transamination, providing nutritional conditions for bacterial survival and reproduction. Certain metabolic processes enhance plant growth and development by producing antibiotics, growth hormones, and antibacterial proteins [46]. Furthermore, significant differences were observed in the relative abundances of these metabolic functions among rhizosphere soil bacteria across different planting years, indicating that the planting years had a significant impact on the metabolic capacity of soil bacteria. Although PICRUSt2 functional prediction can be used to analyze bacterial function, it lacks gene data support, which presents certain limitations. Future research should explore the relationship between rhizosphere bacteria and planting years more comprehensively by integrating metagenomic technologies.

BugBase predictions for rhizosphere soils in different planting years indicated a general upward trend in the abundance of aerobic bacteria, whereas anaerobic bacteria exhibited a downward trend. Both aerobic and anaerobic bacteria in the rhizosphere include members of the phyla *Planctomycetes* and *Acidobacteria*, with varying abundance in different planting years. This variability may be linked to the preference of *Acidobacteria* for anaerobic and microaerophilic environments, as lower pH values in the rhizosphere promote the growth of certain subgroups of *Acidobacteria*. However, *Acidobacteria* cannot survive well in the rhizosphere under nutrient-rich conditions [47]. In contrast, *Planctomycetes* encompass obligate aerobic genera, such as *Mortierella*, and anaerobic ammonium oxidation genera, such as *Candidatus Brocadia*, which can thrive in diverse oxygen environments. Consequently, there were differences in the composition of anaerobic and aerobic bacteria across different years. This study suggests that *Z. bungeanum* is susceptible to pathogen infection during its initial development because of the high proportion of pathogenic bacteria present during the early stages. However, this vulnerability may decrease with increasing planting years, as the number of oxidative stress-tolerant bacteria increases, thereby enhancing the resistance of *Z. bungeanum* to pathogens. It is widely accepted that members of the phyla *Acidobacteria* and *Nitrospirales* are non-pathogenic; hence, it can be inferred that the potentially pathogenic bacteria affecting *Z. bungeanum* originate from the phylum *Proteobacteria* [13]. Research has demonstrated that *Actinobacteria* can withstand pressure and resist pathogenic infections [48]. In this study, *Actinobacteria* emerged as the dominant taxonomic group among the stress-tolerant microorganisms, with its abundance steadily increasing over the planting years, indicating its significant role in facilitating the adaptation of *Z. bungeanum* to environmental stress.

5.4 Relationship between Bacterial Community and Functional Genes in the Rhizosphere Soil of *Z. bungeanum* and Physicochemical Factors

The soil environment, which acts as a habitat for microorganisms, significantly influences microbial diversity and community structure. The determinants of the rhizosphere microbial community can vary across different growth stages or regions of the same plant. Numerous studies have reported that soil physicochemical properties are important factors affecting the composition of soil bacterial communities [49]. For instance, Tang et al. [50] identified key environmental factors affecting the soil bacterial community structure in pecan plantations, including soil type and the pH, available phosphorus content, electrical conductivity, soil moisture and ammonium nitrogen contents. Liao et al. [23] discovered that pH, AP, AK, and polyphenol oxidase activity are the primary factors influencing the rhizosphere soil bacterial community of *Z. bungeanum* in Maoxian County. This study revealed that organic matter, AKP, pH, and AP content are the key factors influencing the bacterial community structure of *Z. bungeanum* rhizosphere soil in Linxia. SOM, as a key energy source, directly shapes microbial community structure and function, and its activity and diversity are regulated by the availability of carbon, nitrogen, and phosphorus [51]. Conversely, soil microbial communities are vital for sustaining soil fertility because they play an essential role in regulating the cycling of carbon, nitrogen and phosphorus in soils. Random forest and RDA also indicated that SOM

was the most significant physicochemical factor affecting the community structure of soil bacteria at the phylum and class levels. Wang et al. [47] found that a decrease in bacterial diversity in the rhizosphere soils of forage on the Qinghai-Tibet Plateau led to a 30.4% reduction in the potential for carbon utilization. Soil pH is one of the main factors affecting the structure and function of soil bacterial communities [52]. There are significant differences in the composition of bacterial communities under different pH conditions [53]. For example, in soils where chemical fertilizers have been applied for a long period of time, changes in pH can lead to the enrichment of specific bacterial taxa, thereby altering the community structure [54]. The significant negative correlation between rhizosphere soil pH and AP content in the present study is consistent with the findings of Ibrahim and Ikhajiagbe et al. [55]. This may be because the effectiveness of phosphorus is reduced under acidic conditions, which in turn affects the rhizosphere bacterial community in peppercorns. Therefore, clarifying the relationship between soil environment and changes in the microbial community structure is crucial for scientifically evaluating the stability and systematicity of soil ecosystems, while also positively impacting *Z. bungeanum* cultivation.

The composition of the soil bacterial community is significantly influenced by the metabolic functions of soil bacteria. Pearson correlation analysis revealed a significant correlation between the changes in the relative abundance of secondary metabolic function genes in the rhizosphere soil bacteria of *Z. bungeanum* and the changes in the relative abundance of Acidobacteriota, Actinobacteriota, and Bacteroidota, which is consistent with the findings of Li et al. [56]. Pold and DeAngelis [57] suggested that soil microorganisms exhibit functional redundancy, indicating that community functions do not change with variations in microbial diversity and structure. However, Liang et al. [58] found that habitat changes lead to increased soil bacterial diversity and alterations in community structure, which play crucial roles in maintaining community functional stability. In the present study, significant differences were observed in the abundance of secondary metabolism functional genes among certain rhizosphere soil bacteria across different planting years, suggesting that planting duration affects the composition of bacterial secondary metabolites. Further investigation is needed to explore the relationship between this observation and changes in the bacterial community structure over time due to planting years. Currently, there is limited research on the intrinsic relationships and mechanisms through which *Z. bungeanum* affects the soil microbial community structure and metabolic functional genes. Future studies should delve deeper into how *Z. bungeanum* shapes the soil microbial community structure and fulfills its specific functions.

6 Conclusions

Planting duration significantly influenced the physicochemical properties, bacterial community composition, and diversity of *Z. bungeanum* rhizosphere soil. Bacterial diversity increased progressively with the stand age. The predominant phyla were Proteobacteria, Actinobacteria, Acidobacteriota, and Chloroflexi. Amino acid and carbohydrate metabolism were the primary metabolic functions of bacteria in *Z. bungeanum* rhizosphere soil, and the relative abundance of functional genes increased significantly as the planting duration increased. The community structure of rhizosphere soil bacteria was mainly influenced by organic matter content, AKP activity, AP content, and pH. Among these factors, organic matter had the most significant impact on the phylum and class levels, as well as metabolic functions. Based on the key role of SOM in shaping bacterial communities and metabolic functions, appropriate cultivation and management measures should be adopted for *Z. bungeanum* plantations with longer planting years, such as applying decomposed compost and covering crops with legumes. By maintaining a certain SOM content, the soil microbial community structure and function of *Z. bungeanum* plantations can be improved, and soil quality can be enhanced to promote the healthy growth of *Z. bungeanum* trees over longer planting years.

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Availability of Data and Materials: Data are contained within the article.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest to report regarding the present study.

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