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Differences in Root Endophytic Bacterial Communities of Chinese Cork Oak (*Quercus variabilis*) Seedlings in Different Growth Years

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Abstract: In forests, seedling renewal is influenced by many environmental factors, including climate change, seed size, wildfires, and ecological factors. It is unclear how different growth years of seedlings affect Chinese cork oak (*Quercus variabilis*) root endophyte communities. In this study, we took a holistic approach, using Illumina sequencing, to study the composition and function of bacterial communities associated with root microorganisms in four *Q. variabilis* seedlings after 1, 2, and 3 years of growth. The bacterial alpha diversity indexes were highest in the second year and lowest in the third year, and age was the decisive factor for the differences found in the root endophytic bacterial communities. Total phosphorus had the greatest effect on bacterial communities. The abundance of beneficial bacteria *Streptomyces* (8.69%) and *Novosphingobium* (4.22%) was highest in the second-year samples, and their abundance decreased by 7.96% and 3.61% in the third year, respectively. Higher levels of plant disease inhibition and metabolism (23.80%) were in the roots of second-year *Q. variabilis* seedlings. The metabolic abundance of carbohydrate was 3.66% lower in the first year and 3.95% lower in the third year compared to the second year. Our results suggest that the structure and function of bacterial communities changed with increasing growth years.

Keywords: *Quercus variabilis*; root endophytic bacterial; bacterial diversity; rhizosphere; growth years

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1. Introduction

Chinese cork oak (*Quercus variabilis*) is one of the most widely distributed trees across Eastern Asia [1], with important ecological and economical value [2]. According to the 9th Chinese National Forest Inventory, the area of China's existing oak-dominated forests is 15.26 million hm^2 and stocking is 1.387 billion m^3, which ranks first in terms of forest area and stocking in the country [3]. More than 90% of the stands are degraded secondary natural forests, with very poor quality because of coppices, subsequent shoot or root regeneration, and historical anthropogenic disturbance [3,4]. Therefore, a more comprehensive understanding of the seedling renewal mechanism of *Q. variabilis* plays an important role in their growth.

In forest ecosystems, seedling regeneration is affected by many environmental factors. For example, the presence of litter may change the predation behavior of rodents, which consequently affects seedling regeneration [5]. Climate change, propagule size, wildfires, and ecological factors such as temperature and soil moisture are all influences on seedling regeneration [6–8]. Moreover, according to the Janzen–Connell (J–C) hypothesis, regeneration seedlings gather around adult trees because of the limits of diffusion, which

makes them prone to resource competition and attack by specialized natural enemies. Additionally, specific host pathogenic bacteria accumulate around the root system [9]. The accumulation of these bacteria leads to a high death rate for seedlings around adult trees [10], but not in the homogeneous seedlings found away from adult trees. Therefore, during early establishment, the J–C effect may be the main reason for the failure of seedling regeneration [11]. Studies showed that the root endophytic microorganisms in soil were connected with seedling regeneration [12–15]. Endophytic bacteria are ubiquitous in most plant species, can latently or actively colonize plant tissues, and develop a range of different relationships with their host plant [16]. For instance, some endophytes produce signals like reactive oxygen species (ROS), which result in the switching of endophytism to either antagonism or mutualism in response to some environmental cues [17]. In addition, some studies have mentioned that the composition and diversity of root endophytic bacteria are related to the rhizocompartment, soil type, and physicochemical properties of soil [18,19]. In addition, different growth times also have a significant impact on the root endophytic microbial community of plants [20]. Therefore, we can hypothesize that the growth time of seedlings affects their bacterial communities, leading to changes in some pathogenic bacteria in the rhizosphere soil, thereby affecting seedling renewal.

However, few studies have focused on the effect of the age of *Q. variabilis* seedlings on the root endophytic microbial community. In this study, we investigated the relationship between root endophytic bacterial communities and seedling age, and the mechanism of plant–microbe–soil interactions. We collected the rhizosphere soil and roots of *Q. variabilis* seedlings under adult trees, naturally grown for one, two, and three years, to explore the changes in soil physicochemical properties and the composition, diversity, and function of bacterial communities. By doing so, we aimed to answer four main questions, including: (1) Will the growth years of Chinese cork oak seedlings affect root endophytic bacterial communities? (2) will changes in root endophytic bacterial communities have an effect on seedling growth? (3) will the growth age of Chinese cork oak seedlings affects the physicochemical properties of the soil? and (4) will changes in the physical and chemical properties of soil have an effect on the growth of seedlings?

2. Materials and Methods

2.1. Study Area and Sample Collection

The sampling site was located in Shimen Mountain (35°71′ N, 117°06′ E), Jining City, Shandong Province, China. The climate is of a north temperate type, with an average annual temperature of around 13 °C, average annual precipitation of 707.1 mm, average annual sunshine of 2406.8 h, and average accumulated active temperature of 4571.9 °C. There are significant seasonal fluctuations in temperature and precipitation throughout the year.

Three groups (*Q. variabilis* seedlings aged 1–3 years: Y1, Y2, and Y3, respectively) of rhizosphere soil samples were randomly selected for the experiment. Two plants per plot (5 m × 5 m) from three random plots were collected for each growth year. Large soil aggregates that were loosely bound to the roots were first removed via shaking, and 30 g of the tightly bound rhizosphere soil [21] was collected separately and mixed into a sample, with the process for each group being repeated four times. The internal tissues of the root were selected for root endophytic bacterial DNA extraction to determine the alpha diversity, beta diversity, and community composition of the root endophytic bacterial community. The soil samples were dried naturally at room temperature for determining the contents of conventional nutrients.

2.2. Soil Physicochemical Properties

The total organic carbon (TOC) content was determined using a TOC-L analyzer (Shimadzu, Japan) by adding 20 mL of 1 mol/L KCl solution to 1 g of air-dried soil samples, leaching for 20 min, and filtering [22]. The soil pH was determined by mixing 5 g of fresh sample with 50 mL of deionized water, shaking for 30 min, and then filtering. The

ammonium (NH_4^+)-nitrogen (N) and nitrate (NO_3^-)-N concentrations were determined using an AQ2 Discrete Analyzer (SEAL Analytical, Hanau, Germany) with a 2 mol/L KCl solution (1:10 *w*/*v*) [23]. The total phosphorus (TP) content was determined using perchloric acid–sulfuric acid digestion and via the Mo–Sb colorimetric method [24].

2.3. DNA Extraction and PCR Amplification

The root samples were cleaned using ultrasonic waves 3 times and disinfected with sodium hypochlorite 2 times for DNA extraction. Subsequently, the quality of the DNA extract was evaluated using 1% agarose gel and a NanoDrop 2000 UV–vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Fragments of 16S rRNA genes of bacteria were amplified using a PCR thermocycler (ABI 9700, USA) with primer pairs 799F (5′-AACMGGATTAGATACCCKG-3′) and 1193R (5′-ACGTCATCCCCACCTTCC-3′). The amplified PCR products were extracted using 2% agarose gel and purified using the AxyPrep DNA gel extraction kit (Axygen, Union City, CA, USA), followed by paired-end sequencing using the MiSeq PE300 platform (Illumina, San Diego, CA, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) [25]. The sequences obtained have been submitted to the NCBI SRA database under the accession number PRJNA 974679.

2.4. Bioinformatics and Statistical Analysis

The raw sequencing reads were demultiplexed, quality-filtered using fastp version 0.20.0 and merged using FLASH version 1.2.11 [26]. Briefly, sequences were discarded if they contained any ambiguous base, had more than two mismatches to the primers, one mismatch to the barcode sequence, a minimum sequence length of 200 bp, or an average quality score of 30. Sequences were clustered into amplicon sequence variants (ASVs) with similarities of 97% using UPARSE version 7.1, and chimeric sequences were identified and removed [27]. The taxonomy of ASV representative bacterial sequences was analyzed using RDP Classifier version 2.2 against the 16S rRNA database (Silva v132, http://www.arb-silva.de, accessed on 1 January 2022) [28]. Alpha diversity indexes (Ace, Shannon, Simpson, Shannoneven, Simpsoneven, and Chao) were calculated in the Phyloseq R package. Beta diversity was estimated via non-metric multidimensional scaling (NMDS) analysis using the Bray–Curtis dissimilarity method. The distribution of the dominant phyla of samples at different phases was visualized using Circos [29].

The statistical significance of soil physicochemical properties and bacterial alpha diversity indices was tested using a one-way ANOVA and a paired comparison of treatment means analyzed via Duncan's test at $p < 0.05$. All data were calculated with IMB SPSS 19 (IBM Corporation, New York, NY, USA). The relationships between the bacterial microbial communities and physicochemical properties (including pH value, TOC, TP NH_4^+-N, and NO_3^--N) were evaluated via distance-based redundancy analysis (db-RDA) using the R Vegan package. Furthermore, PICRUSt2 was used to identify the bacterial metabolic function based on the KEGG database [30].

3. Results

3.1. Physicochemical Properties

The soil pH and concentrations of TOC, TP, NH_4^+-N, and NO_3^--N are shown in Table 1. Soil pH and TP concentration decreased significantly with increased *Q. variabilis* age. However, the contents of TOC, NH_4^+-N, and NO_3^--N in the rhizosphere soil of different ages fluctuated to a certain extent, but their contents were highest for Y2 and lowest for Y1.

Table 1. Soil properties of *Q. variabilis* rhizosphere in different growth years.

	Y1	Y2	Y3
pH	5.810 ± 0.334 ^{b}	5.547 ± 0.050 ^{ab}	5.458 ± 0.041 ^{a}
TP	0.029 ± 0.005 ^{b}	0.027 ± 0.001 ^{b}	0.016 ± 0.001 ^{a}
NH_4^+-N	0.008 ± 0.001 ^{a}	0.014 ± 0.005 ^{b}	0.010 ± 0.001 ^{ab}
TOC	4.997 ± 0.476 ^{a}	7.725 ± 0.617 ^{c}	6.379 ± 0.418 ^{ab}
NO_3^--N	0.625 ± 0.056 ^{a}	1.619 ± 0.056 ^{c}	1.052 ± 0.303 ^{b}

Note: $n = 6$. Different letters indicate significant differences at $p < 0.05$. TP: total phosphorus; NH_4^+-N: ammoniacal nitrogen; TOC: total organic carbon; NO_3^--N: nitrate nitrogen; Y1: annual *Q. variabilis;* Y2: biennial *Q. variabilis;* Y3: triennial *Q. variabilis*.

3.2. Alpha Diversity

After the selection and chimera analysis of the ASVs, there were 1023015 raw sequence reads, including 974,007 high-quality sequences assigned to 125 ASVs (Table S1). Based on a similarity cutoff of 97%, Good's coverages ranged within 96.0%–99.7% (Figure 1), indicating that the number of reads was sufficient to represent the bacterial diversity in all samples.

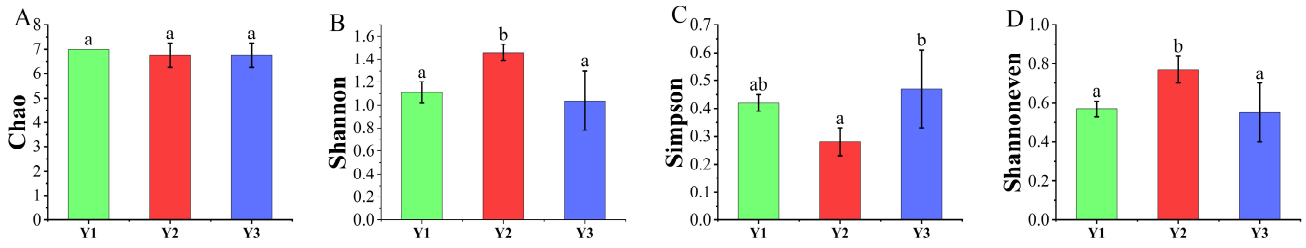

Figure 1. Alpha diversity of *Q. variabilis* seedlings grown in different growth years. (**A**) Chao index; (**B**) Shannon index; (**C**) Simpson index; (**D**) Shannoneven index. Different letters indicate significant differences at $p < 0.05$.

Bacterial alpha diversity indices (including Shannon, Simpson, and Shannoneven) are shown in Figure 1. The Y2 had the highest Shannon and Shannoneven indices and Y3 had the lowest; Y2 had the lowest Simpson indices and Y1 had the highest. Thus, Y2 and Y3 seedlings had the highest and lowest bacterial alpha diversity in the roots, respectively.

3.3. Beta Diversity

The result of the Bray–Curtis NMDS is shown in Figure 2. The root endophytic bacterial community of *Q. variabilis* seedlings in different growth years clustered into different groups, indicating differences in bacterial community structure among growth years.

The first axis of the NMDS (i.e., NMDS1) generally separated the samples into two groups based on age: Groups I and II were similar and differed from Group III. Similarly, NMDS2 separated samples into two groups based on age: Groups II and III were similar and differed from Group I.

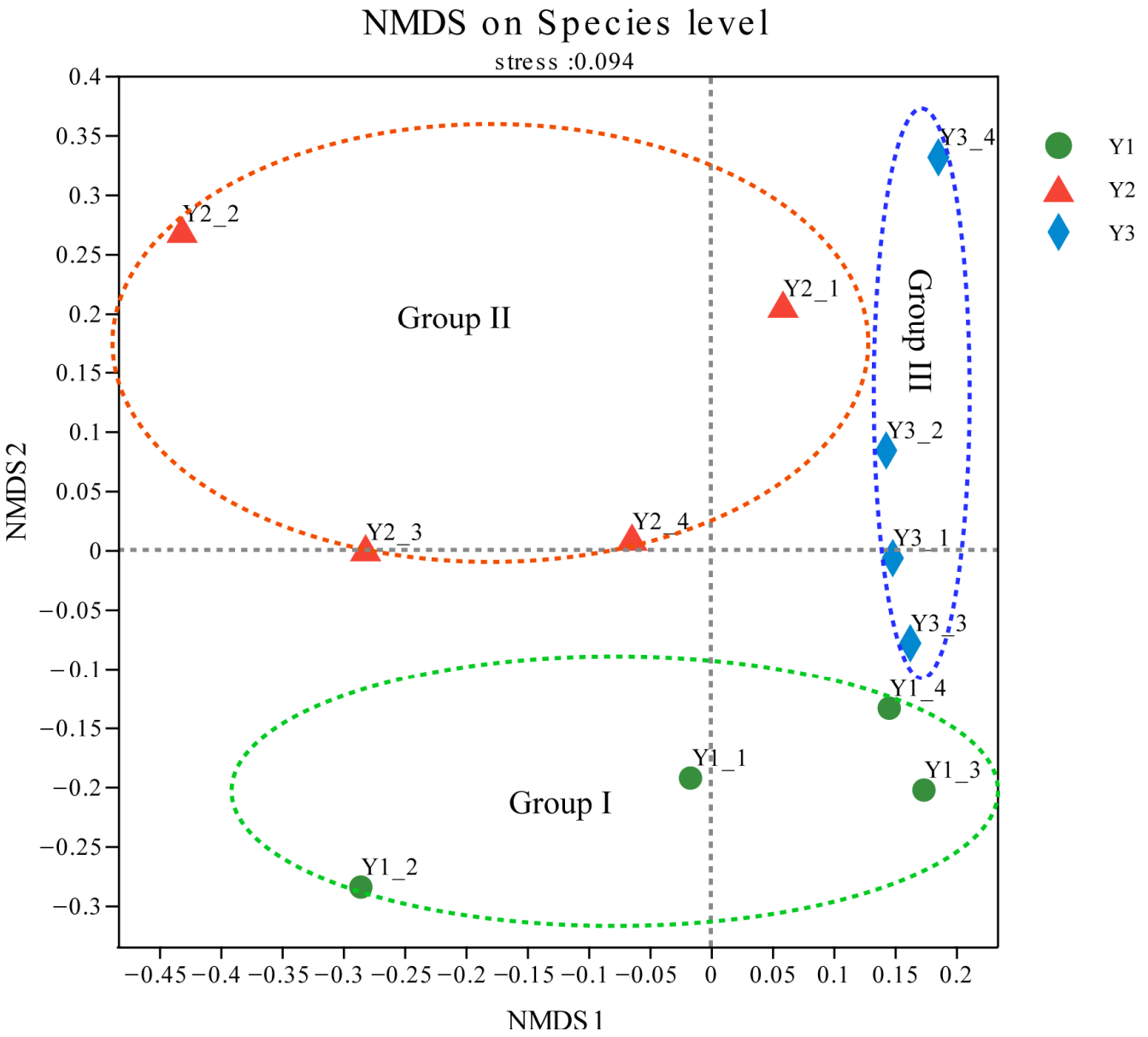

Figure 2. NMDS analysis of root endophytic bacteria of *Q. variabilis* in different growth years.

3.4. Environmental Factors Influencing Bacterial Community Structure

The RDA result is shown in Figure 3. The first two axes (i.e., CAP1 and CAP2) together explained 41.17% (CAP1, 24.24%; CAP2, 16.93%) of the variance in bacterial communities of *Q. variabilis* in different growth years. The difference between the Y2 and Y3 groups was mainly shown on CAP1, and the difference between Y2 and Y1 was mainly shown on CAP2. The NO_3^--N concentration had a large projection on CAP1, and soil pH and concentrations of TP, TOC, and NH4+-N all had large projections on CAP2. The concentrations of NO_3^--N ($p = 0.076$) and TP ($p = 0.045$) had a great effect on the variance in the bacterial community and may be major indicators of community differences. In Y2 samples, the bacterial community was positively correlated with the TOC, TP, NH_4^+-N, and NO_3^--N, but negatively correlated for Y1 and Y3.

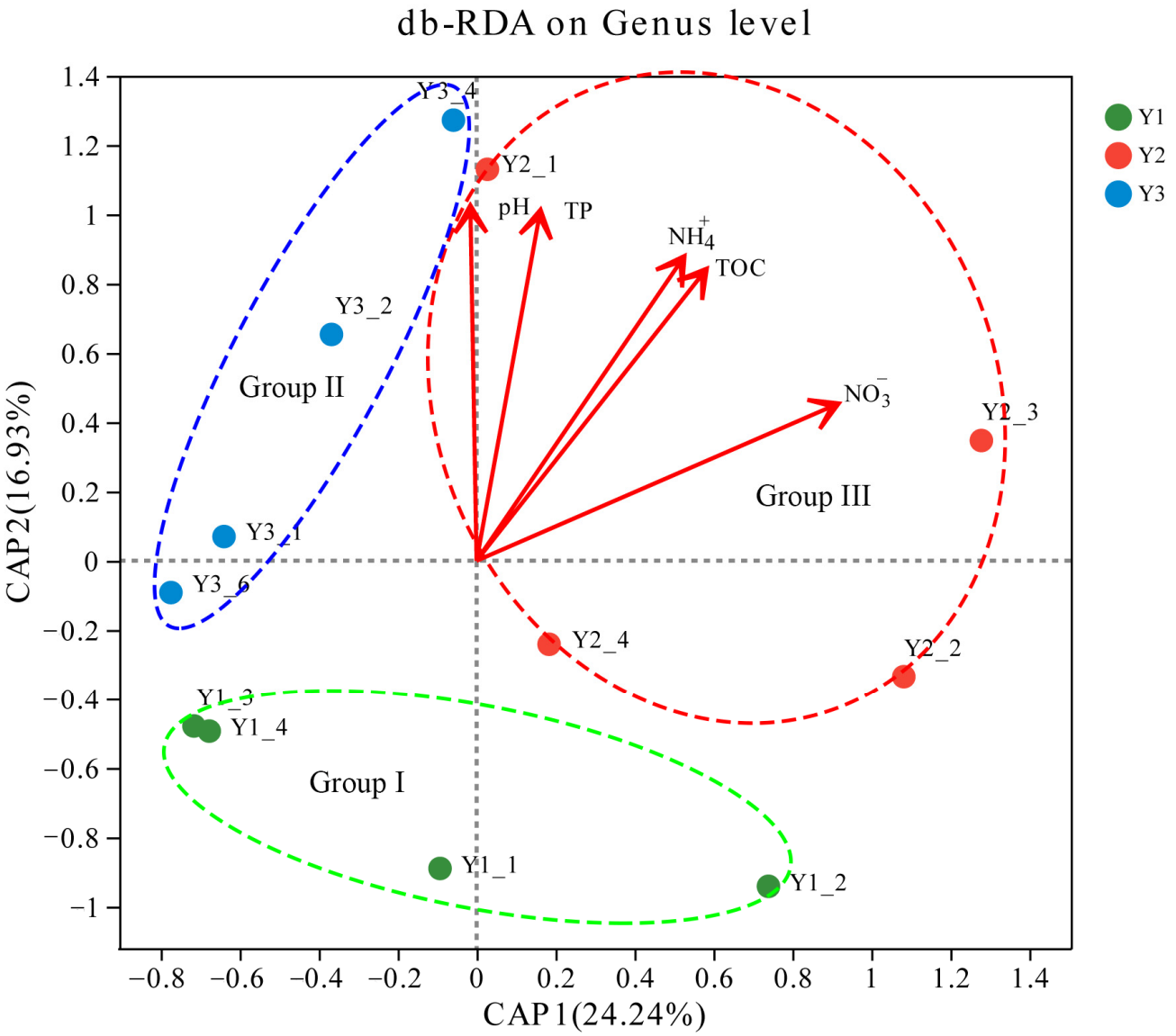

Figure 3. db-RDA of bacterial communities and environmental factors for individual samples. Environmental factors include pH, TP (total phosphorus), NH_4^+ (ammoniacal nitrogen), NO_3^- (nitrate nitrogen), TOC (total organic carbon).

3.5. Bacterial Community Structure

Bacterial community composition at both the phylum and genus levels is shown in Figure 4. The top three dominant phyla with obvious changes in relative abundance were Proteobacteria (75.17%–79.62%), Actinobacteriota (14.56%–17.47%), and Acidobacteriota (0.73%–3.73%). The relative abundance of Proteobacteria decreased with the age of *Q. variabilis:* Y2 was 0.76% lower than Y1, and Y3 was 0.37% lower than Y2.

At the genus level, the top three dominant genera were *Bradyrhizobium* (5.24%–8.97%), Streptomyces (0.34%–8.69%), and *Acidothermus* (0.15%–5.04%) (Figure 4). Differences in the root endophytic bacterial community of Y2 *Q. variabilis* were more obvious. The relative abundances of *Acidothermus* (0.15%), *Sphingomonas* (0.75%), and *Acetobacter* (0.41%) were lowest in the Y2 rhizosphere soil samples, while relative abundances of *Streptomyces* and *Novosphingobium* were highest in Y2. Additionally, the relative abundance of *Mycobacterium* decreased with the age of the *Q. variabilis*: Y2 was 0.23% lower than Y1, and Y3 was 0.24% higher than Y2. In addition, the relative abundance of *Cryptosporangium* progressively increased with the *Q. variabilis* age, reaching 1.27% in Y3.

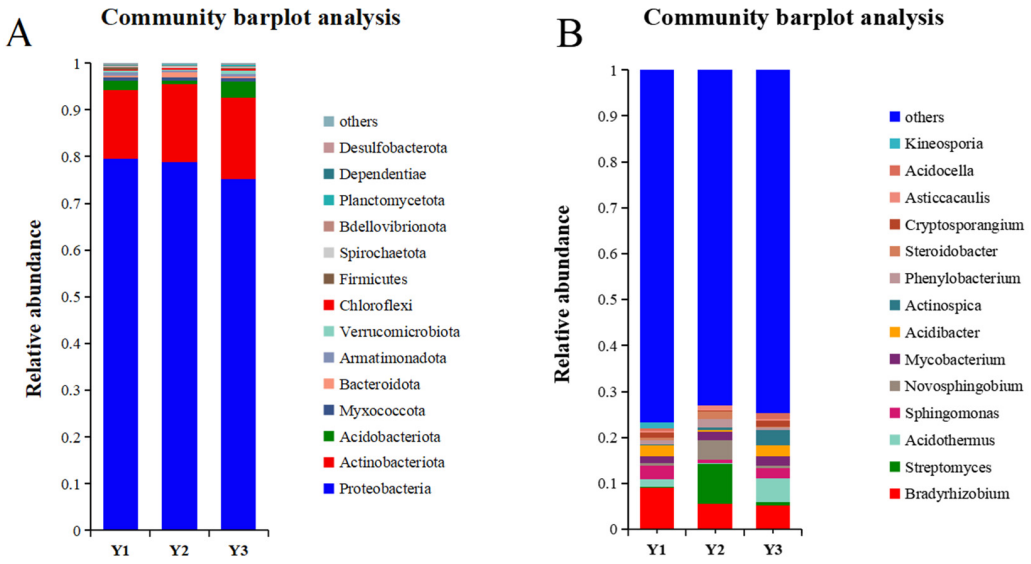

Figure 4. Composition of root endophytic bacterial community of *Q. variabilis* in different growth years. (**A**): Relative abundances < 0.1% were indicated as "others" at the phylum level. (**B**): Relative abundances < 1% were indicated as "others" at the genus level.

3.6. Function Analysis

Based on the KEGG pathway database, the bacterial functions were predicted using PICRUSt2 (Figure 5). The function predictions included six pathways for metabolism: five for organismal systems and one for cellular processes. Carbohydrate metabolism (8.33%–8.91%) was the main metabolic pathway in the root endophytic bacterial community of *Q. variabilis* of different ages, followed by amino acid metabolism (8.17%–8.35%). This indicated that carbohydrate as the major carbon (C) and energy source was utilized in microbial metabolism. In addition, the functions of metabolisms, organismal systems, and cellular processes showed a similar tendency in the three groups. In Y2 samples, the functions of carbohydrate metabolism (8.91%), amino acid metabolism (8.35%), lipid metabolism (2.62%), biosynthesis of other secondary metabolites (1.48%), metabolism of terpenoids and polyketides (1.26%), glycan biosynthesis and metabolism (0.99%), transport and catabolism (0.37%), the nervous system (0.20%), substance dependence (0.06%), immune disease (0.04%), and the digestive system (0.04%) were higher than those for Y1 and Y3 (Figure 5).

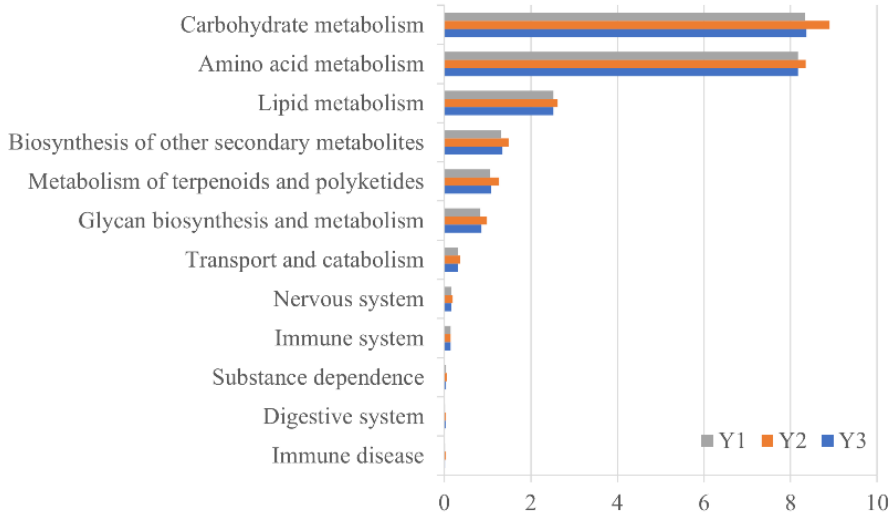

Figure 5. The relative abundance of KEGG-assigned functional categories in the groups.

4. Discussion

4.1. Effects of Seedling Age

Soils are made up of highly diverse microbial communities that mediate a variety of important processes and functions, and these microbial communities have been reported to change to some extent depending on time scales [31]. In our study, NMDS results also showed that the root endophytic bacterial communities of *Q. variabilis* seedlings could be divided into three major groups (Y1, Y2, and Y3), and the results showed that growth years (age) were the decisive factor in the difference in root endophytic bacterial communities. Many studies have shown that root endophytic bacterial diversity in various crops shows different trends at different growth stages, such as the trend of an initial rise followed by a decline or an overall trend of gradually decreasing or increasing with the growth stage [6]. In this study, the alpha diversity indicator (Shannon) of different sampling periods increased first and then decreased. This is consistent with some previous findings but inconsistent with others [32]. The inconsistencies may be related to differences in planting systems, plant characteristics, soil type, soil temperature and humidity, and climatic characteristics at the study site.

4.2. Changes Favorable to Seedlings

In this study, the bacterial diversity of the root of *Q. variabilis* seedlings in Y2 was higher than in Y1, possibly because during the first two years the seedling growth gradually improved, and the interaction and communication with root endophytic bacteria were also better, thus promoting the diversity of root endophytic bacteria. However, whether seedling growth and physiological indicators were better in Y2 than Y1 remains unclear and should be verified in future studies.

Changes in soil environmental factors in Y2 seedlings may indicate healthier seedlings at this stage. The bacterial alpha diversity is strongly influenced by soil environmental factors [33]. Previous studies have shown that soil N can promote organic matter decomposition in soil by microbes, thereby increasing the diversity of bacterial communities [34]. In our study, the bacterial Shannon index and contents of NH_4^+-N and NO_3^--N were all highest in Y2 samples, consistent with previous results [35]. Addition of N can alleviate soil microbial C restriction by altering soil C availability and the C:N ratio, leading to more diverse microbial communities [36], which also explains why the carbohydrate metabolism was most vigorous when the soil had the highest NH_4^+-N and NO_3^--N content in Y2. In previous studies, the bacterial Shannon index increased with increasing *p* levels [37], but our results differed. This may be because the content of NH_4^+-N and NO_3^--N was much higher than that of TP. Overall, balanced nutrients are of great significance for maintaining the diversity of soil bacterial communities [38].

The plant roots and the surrounding soil environment, referred to as the rhizosphere, have diverse and dynamic microbial communities that come into direct contact with the roots and can influence the physiological activity of plants [39]. The rhizosphere microbial composition is essential for achieving sustainable ecosystem functioning [40]. In our study, the increase in beneficial bacteria in the seedling root for Y2 may also indicate healthier seedlings at this stage. For example, the abundance of *Streptomyces* (8.69%) and *Novosphingobium* (4.22%) was highest in the Y2 rhizosphere soil samples, and in Y3, their abundances decreased by 7.96% and 3.61%, respectively. *Streptomyces* can help some bacteria thrive in the environment by producing secondary metabolites [40]. *Novosphingobium* can promote plant growth or break down insoluble matter in rhizosphere soil via symbiosis with plants [41].

4.3. Changes Harmful to the Q. variabilis Seedlings

However, by the third year, root endophytic bacterial diversity decreased, most likely because seedling growth was affected by certain harmful bacteria, with some pathogenic bacteria accumulating around the root of *Q. variabilis*. For example, the relative abundance of Mycobacterium and Cryptosporangium increased with *Q. variabilis* age. Studies

have shown that Mycobacterium has a negative effect on the microreproduction of Pinus sylvestris [42]. Cryptosporangium can cause leaf and shoot blight and has shown a high ability to inhibit eucalyptus growth [43]. Therefore, increases in Mycobacterium and Cryptosporangium may be important obstacles for *Q. variabilis* seedlings in the process of forest regeneration, and may also be important factors in the J–C hypothesis. In future studies, pure culture techniques should be used to screen for these microorganisms and verify their pathogenicity on healthy *Q. variabilis* seedlings.

Furthermore, we found that continuous cultivation could reduce the abundance of beneficial bacteria in the soil [44,45]. Therefore, some beneficial bacteria were less abundant for Y3 than Y2, which may also have led to a decrease in root endophytic bacterial diversity for Y3. For example, with increased age, the abundance of beneficial Proteobacteria and Firmicutes decreased with the duration of continuous cropping, similar to previous studies [44]. Proteobacteria are involved in N fixation, decomposition of organic matter, and promotion of plant growth [46]. The decrease in the relative abundance of phylum Proteobacteria with *Q. variabilis* age is inconsistent with results of previous studies [47] and may be related to environmental factors such as soil pH [48]. Therefore, the decrease in the abundance of beneficial bacteria with age suggests that this is a key barrier to maintaining the relative abundance of beneficial bacteria in the soil to promote plant growth and resistance to biological stresses.

4.4. Bacterial Community Responses to Environmental Conditions

Soil physicochemical properties such as pH, TOC, total N, or TP are important drivers of bacterial community structure [49,50]. The RDA results showed that P was the key environmental variable determining root endophytic bacterial communities. However, studies have shown that changes in the composition of microbial communities are not directly caused by an increase in P [51]. Increasing P content in soil can increase soil pH, which indirectly affects the composition of the soil microbial community [51]. In this study, the TP content in the soil decreased with the *Q. variabilis* age, and soil pH was positively correlated with the P content. Since the pH value decreased annually, this led to a progressive decrease in beneficial Proteobacteria, consistent with previous studies [52]. Additionally, we found that the relative abundance of Firmicutes (0.54%) and TP were highest for Y1, and the relative abundance of Firmicutes decreased to some extent as the P content decreased, consistent with previous research [37]. In addition, a study claimed that the TP concentration strongly affected microorganisms involved in P metabolism and thus the entire bacterial community [50]. Overall, the decrease in P content in soil directly or indirectly affected the composition of soil microbial communities, and thus affected regeneration of *Q. variabilis* seedlings. Therefore, the amount of P in the soil may also be an important factor in influencing the J–C hypothesis, but further validation is required.

4.5. Effects of Age on Bacterial Community Function

Changes in the structure and composition of bacterial communities often lead to changes in metabolic capacity, biodegradation, disease inhibition, and other functions [53]. For example, Proteobacteria and Actinobacteriota are the key factors in bacteriostatic effects on soil and contain more genes encoding enzymes involved in carbohydrate metabolism. In this study, the highest abundances of Proteobacteria (78.89%) and Actinobacteriota (16.61%) were for the Y2 *Q. variabilis* root endophytic microorganisms; the soil bacteriostatic effect was relatively high, and carbohydrate metabolism (8.33%–8.91%) was also the most vigorous. It has been shown that the C cycle is a central part of bacterial metabolism in soil [54]. In addition, soil physicochemical properties can significantly affect the function of bacterial communities by changing the composition of bacterial communities or affecting bacterial activity [55]. Therefore, in future research, we should further investigate the relationships among bacterial community composition and function and soil physicochemical properties to determine the beneficial growth-promoting bacteria and pathogenic bacteria unfavorable to the growth of *Q. variabilis* seedlings.

In this study, the Y2 *Q. variabilis* rhizosphere soil had a higher proportion of amino acid and carbohydrate metabolism. As indicated by the above-mentioned result, age affected the important role of soil bacteria in the metabolism and synthesis of amino acids and carbohydrates in the *Q. variabilis* rhizosphere. This may be because age affected *Q. variabilis* growth to some extent by affecting bacterial diversity in the rhizosphere soil, regulating the function of soil bacteria [56]. Additionally, increased cell transport may contribute to the survival and colonization of root-specific bacterial pathogens [22], which may also be an important factor in the J–C hypothesis. In future studies, the effect of increased cell transduction abundance on two pathogens, *Mycobacterium* and *Cryptosporangium*, should be further studied. The prediction of bacterial function is helpful to further verify the changes in bacterial community structure in rhizosphere soils and indicate the impact of growth years on rhizosphere soil ecology.

5. Conclusions

We found that growth year was an important factor leading to differences in root endophytic bacterial communities of *Q. variabilis*. This also had a significant effect on soil physicochemical properties, thus affecting the diversity of microorganisms in the *Q. variabilis* rhizosphere, resulting in the highest bacterial alpha diversity for Y2 seedlings. Additionally, some beneficial bacteria initially increased with age and later decreased. This may indicate that they were important players in the seedling renewal process. However, the relationship between *Q. variabilis* and bacterial communities needs further study, and pure culture and pot experiments should be used for further validation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14071489/s1. Table S1: Details of ASVs in each sample.

Author Contributions: Conceptualization, W.S., J.Z. and Y.C.; methodology, Y.K. and D.H.; software, D.H.; validation, D.H., Y.X. and Y.C.; formal analysis, X.Y.; investigation, B.L.; resources, W.S.; data curation, W.S.; writing—original draft preparation, W.S.; writing—review and editing, D.H.; visualization, D.H.; supervision, Y.C.; project administration, J.Z. and W.S.; funding acquisition, W.S. and G.Y. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available in the article. The sequences obtained have been submitted to the NCBI SRA database under the accession number PRJNA 974679.

Conflicts of Interest: The authors declare no conflict of interest.

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