# EFFECTS OF GENETIC RELATIONSHIPS OF DIFFERENT OAKS (*QUERCUS SPP.*) ON THEIR RHIZOSPHERE BACTERIAL COMMUNITIES

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(Received 11th Dec 2024; accepted 27th Feb 2025)

**Abstract.** There is a complex symbiotic relationship between plants and rhizosphere bacteria: the rhizosphere exudates of plants can affect the rhizosphere bacterial communities. However, the effects of the genotypes of different *Quercus* species on rhizosphere bacteria remain unclear. In the present study, we explored the diversity and abundance of rhizospheric bacterial communities in five *Quercus* species *Quercus aliena* (AS), *Quercus dentate* (DS) (Wagner et al., 2016), *Quercus mongolica* (MS), *Quercus variabilis* (VS), and *Quercus wutaishanica* (WS). The rhizosphere bacterial alpha diversity indices were significantly different, with AS showing the highest and VS the lowest diversity. In addition, Proteobacteria (28.7%–38.9%) and Firmicutes (11.2%–23.9%) were the two dominant phyla with apparent changes in relative abundance. Moreover, higher levels of metabolism were observed in AS (17.23%), followed by DS (16.61%), and the major metabolic function of bacteria in AS and DS was higher than that in MS, WS, and VS. The genetic relationship between *Q. aliena* and *Q. dentata* was relatively close, and the relative abundance of their rhizosphere bacteria (such as Proteobacteria and Bacteroidota) was similar. Conversely, the relative abundance of rhizosphere bacteria (such as Bacteroidota and Acidobacteria) varied greatly owing to the genetic relationship of *Q. variabilis* compared to the other relatively distant species. Therefore, our results suggest that the diversity and relative abundance of rhizosphere bacteria correlate with genetic relationships among different *Quercus* species.

**Keywords:** *Quercus species, rhizosphere bacteria, genetic relationships, bacterial diversity, rhizosphere microbes*

## Introduction

The genus *Quercus* has diversified into numerous species in Asia, with the highest diversity observed at 15–30°N, and most recent estimates place the number of *Quercus* species at approximately 500 (Kremer and Hipp, 2019). However, their relative taxonomic classification is challenging because of the high number of species and their wide geographical distribution (Di Marco et al., 2023). In addition, *Quercus spp.* hold great economic importance and ecological value as a major source of high-quality timber in the Northern Hemisphere (Mölder et al., 2019). However, the reduction in *Quercus* biodiversity is becoming a matter of global concern, and several *Quercus* species have been included in the International Union for Conservation of Nature Red List of Threatened Species (Bellusci et al., 2023), largely because of unsustainable harvesting, deforestation for land use changes, and livestock grazing (Wang et al., 2022b; Di Marco et al., 2023). Moreover, because *Quercus spp*. have a high migration capacity, divergence rate, and hybridization propensity, their evolutionary pattern is extremely intricate. It continues to change rapidly, making it difficult to resolve fully (Bellusci et al., 2023). In this study, we focus on exploring the impact of the genetic relationships among different *Quercus spp.* on their rhizosphere bacterial communities. The aim is to reveal the role of genetic diversity in shaping the structure of rhizosphere microbial communities and provide new insights for the biodiversity conservation of *Quercus spp.*

Plants and rhizosphere microbes have evolved various interactions in the long process of evolution such that plants can help their rhizosphere microbes adapt to changes in their environment (Philippot et al., 2013); rhizosphere microorganisms can also influence plant growth and development processes (Mommer et al., 2016; Sha et al., 2023). Therefore, understanding the community composition and species diversity of the rhizosphere microbiome associated with plants is essential to maintaining a healthy rhizosphere environment that improves plant health and productivity (Mendes et al., 2013; Saleem et al., 2019). However, the interactions between the plant and rhizosphere microbes in the soil are complex. For example, biotic factors in the soil (growth age of plants and invading pathogenic microorganisms) and abiotic factors (soil composition, physicochemical properties, and climatic conditions) affect the composition, diversity, structure, and function of rhizosphere microorganisms (Deniau et al., 2017; Xia et al., 2022; Liu et al., 2023; Chinta and Araki, 2023). For instance, the researchers demonstrated in poplars that stand genetic diversity may influence not only soil microbial communities, but also soil processes (Schweitzer et al., 2011). It is proved that plant genotype has a great influence on soil microbial community. In some studies, the effect of different genotypes of the same plant species on the composition of soil microbial communities differed significantly (Fernández et al., 2020). Studies have shown that different maize plant genotypes can produce different rhizosphere exudates, resulting in different carbon allocation strategies and changes in rhizosphere microbial communities (Aira et al., 2010). Other studies have shown that a plant's genotype can determine its rhizosphere exudates, which may affect essential ecosystem processes such as organic matter decomposition and nutrient cycling in rhizosphere soils, thereby affecting the composition and abundance of rhizosphere microorganisms (Brolsma et al., 2017). The heritability of the microbiome determines whether it can evolve (as part of the host’s extended phenotype) in response to selection on host plants (Bordenstein and Theis, 2015). Because the plant microbiome can enhance stress tolerance, disease resistance, and nutrient uptake, understanding the factors governing the recruitment and assembly of rhizosphere communities is key to understanding plant evolution and improving breeding efficiency (Philippot et al., 2013).

However, few studies have focused on the effect of *Quercus* spp. genetic relationships on rhizosphere bacterial abundance and community. In this study, we investigated the correlation between rhizosphere bacteria and *Quercus* spp. genotype. We collected rhizosphere soil from five different *Quercus* species (*Quercus aliena*, *Quercus dentate*, *Quercus mongolica*, *Quercus variabilis*, and *Quercus wutaishanica*) to explore the changes in the abundance and diversity of bacterial communities. Additionally, we studied their genetic relationships by constructing a phylogenetic tree using the internal transcribed spacer (ITS) sequences. In doing so, we aimed to answer three main questions:

1) Will the genetic relationships of *Quercus* affect the rhizosphere bacterial communities? 2) How do the rhizosphere bacterial communities of different *Quercus* spp. change? 3) What are the possible ways in which the genetic relationships of *Quercus* spp. affect the rhizosphere bacterial communities?

## Materials and methods

### Study area and sample collection

The soil samples for the experiment were collected from Shimen Mountain (E 117°5′15″–117°7′33″, N36°46′6″–36°47′9″), Qufu City, Shandong Province. The climate is north-temperate. The average annual temperature is approximately 13℃. The average annual precipitation is 707.1 mm, the average annual sunshine is 2406.8 hours, and the average accumulated active temperature is 4571.9℃ (Sha et al., 2023). There are significant seasonal fluctuations in temperature and precipitation. In order to ensure that the *Quercus* spp. rhizosphere bacteria are recruited by the bacteria in the seed, and to avoid the interference of other bacteria in the soil, we sterilized the soil three times at 121℃ for 20 min each before use.

Seeds of different *Quercus* species were collected from the Jining Forestry Protection and Development Service Centre. Seeds were stratified at 4℃ and surface sterilized by soaking in 2.5% NaOCl for 5 min, followed by five rinses with sterile distilled water before sowing. The seeds were sown in plastic trays filled with autoclaved vermiculite in mid-April 2023. Conditions during germination were 12 h light at 22℃. One month after germination, seedlings were transferred to 6 × 6.5 × 23 cm plastic pots with a 2 mm diameter mesh at the base, filled with a sterile peat and sand mixture. Ten experimental replicates were established for each *Quercus* species because of the non-negligible risk of sapling death during the experiment. Fifty pots were used in this experiment.

The experiment lasted for six months. The pots were laid out at 0.8-m intervals in a square grid, with a random arrangement (monthly change) of treatments and species. The pots were not shielded from sunlight. All pots were watered with the same volume of sterile water (0.25 L per pot) when the precipitation was insufficient (mainly in spring and summer). The pots were checked once a week, and emerging weeds were removed manually. Slow-growing, non-rooting plants (bryophytes) were not removed to avoid unnecessary soil disturbance.

At the end of the experiment in September 2023, we selected the five *Quercus* species of rhizosphere soil samples, and there are *Q. aliena* (AS), *Q. dentate, Q. mongolica* (MS), *Q. variabilis* (VS) *and Q. wutaishanica* (WS) respectively, because they are the major *Quercus* species in north and northeast China (Wang et al., 2022b). Six pots containing seedlings with similar growth were selected from the 10-pot experimental replicates for each *Quercus* species. Large soil aggregates loosely bound to the roots were first removed by shaking, and 30 g of the tightly bound rhizosphere soil was collected separately and mixed into a representative sample (Zhou and Fong, 2021). The process was repeated four times for each group. Rhizosphere soil was used for bacterial DNA extraction to determine alpha diversity, beta diversity, and community composition of the rhizosphere bacterial communities (Sha et al., 2023).

### *DNA extraction, PCR amplification and high-throughput sequencing*

Total DNA was extracted from the soil using a Power Soil DNA Isolation Kit (MOBIO Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Subsequently, the quality of the DNA extract was evaluated on a 1% agarose gel using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, USA). Fragments of 16S rRNA genes of bacteria were amplified using a PCR thermocycler (ABI 9700, USA) with primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Zhang et al., 2020). The amplified PCR products were extracted using a 2% agarose gel and purified using the AxyPrep DNA gel extraction kit (Axygen, USA), followed by paired-end sequencing using the MiSeq PE300 platform (Illumina, USA) according to standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) (Yang and Song, 2019). The obtained sequences were submitted to the NCBI SRA database under accession number PRJNA1047939.

### *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(Magoc and Salzberg, 2011). Sequences were clustered into operational taxonomic units (OTUs) with a similarity of 97% using UPARSE version 7.1, and the chimeric sequences were identified and removed (Edgar, 2013). The taxonomy of representative amplicon sequencing variants (ASV) bacterial sequences were analyzed using RDP Classifier version 2.2 against the 16S rRNA database (Silva v132, http:// www. arb- silva. de) (Nilsson et al., 2019). Alpha diversity indices, including the Sobs, Ace, and Shannon indices, were calculated using the Phyloseq R package (McMurdie and Holmes, 2013). Beta diversity was estimated by non-metric multidimensional scaling (NMDS) analysis based on the Bray–Curtis similarity matrix and visualized using CANOCO 5.0. The ITS sequences of *Q. aliena, Q. dentate, Q. mongolica, Q. variabilis,* and *Q. wutaishanica* were searched in NCBI. The sequences were aligned using the BLAST search program, and a phylogenetic tree was constructed using MEGA version 11.0 and the neighbor-joining method with a bootstrap value of 1000 replicates. Furthermore, PICRUSt2 was used to identify the bacterial metabolic functions based on the KEGG database (Kanehisa et al., 2012).

## Results

### *Phylogenetic tree construction of ITS sequence*

The NCBI Blast retrieval system was used to analyze gene sequence homology. The results of the ITS sequence homology comparison showed that three main lineages emerged from the root: one major clade formed by AS (*Q. aliena*) and DS (*Q. dentate*) (74% bootstrap support), and its sister group formed by MS (*Q. mongolica*, 63% bootstrap support) (*Figure 1*). The second major clade was formed by WS (*Q. wutaishanica*), and the third clade was composed of VS (*Q. variabilis)*. The above results showed that *Q. aliena* and *Q. dentate* have a high degree of similarity, *Q. mongolica* and *Q. wutaishanica* have a high degree of similarity, and, secondarily, *Q. mongolica* and *Q. wutaishanica* have a high similarity with *Q. variabilis.*

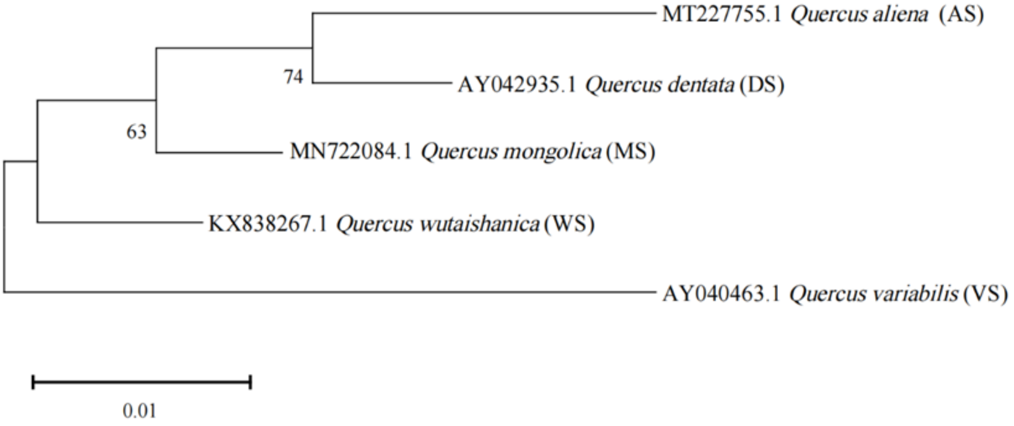

***Figure 1.*** *Phylogenetic tree constructed using the ITS sequences*

### *Alpha diversity*

After selection and chimera analysis of the OTUs, 1,814,746 high-quality sequences were assigned to 12420 non-singleton OTUs, resulting in the classification of 1980 taxa at the species level. Based on a similarity cutoff of 97%, Good's coverage for all samples was higher than 99%, which indicated that the number of reads was sufficient to represent the bacterial diversity in all samples (*Table 1*).

***Table 1.*** *Alpha diversity of rhizosphere bacteria of five different Quercus species*

| Estimators | Sobs | Ace | Chao | Shannon | Simpson | Good's coverage |
|---|---|---|---|---|---|---|
| AS | 511.67 ± 43.83 b | 518.71 ± 47.34 b | 516.81 ± 46.67 b | 5.52 ± 0.08 b | 0.01 ± 0.00 a | 1.00 ± 0.00 a |
| DS | 478.67 ± 31.07 ab | 482.98 ± 31.47 ab | 481.25 ± 31.29 ab | 5.41 ± 0.08 ab | 0.01 ± 0.01 ab | 1.00 ± 0.00 ab |
| MS | 507.67 ± 15.92 ab | 517.11 ± 16.38 b | 515.01 ± 17.36 b | 5.44 ± 0.09 ab | 0.01 ± 0.02 ab | 1.00 ± 0.00 a |
| VS | 476.67 ± 55.73 ab | 486.38 ± 60.26 ab | 485.48 ± 60.79 ab | 5.31 ± 0.12 a | 0.01 ± 0.03 b | 1.00 ± 0.00 a |
| WS | 477.50 ± 24.06 a | 449.01 ± 25.84 a | 448.10 ± 25.08 a | 5.45 ± 0.06 ab | 0.01 ± 0.04 a | 1.00 ± 0.00 b |
| P-value | 0.037 | 0.031 | 0.034 | 0.013 | 0.004 | <0.001 |

Note: n = 6. Different letters indicate significant differences at $P < 0.05$. AS: Q. aliena rhizosphere soil; DS: Q. dentate rhizosphere soil; MS: Q. mongolica rhizosphere soil; VS: Q. variabilis rhizosphere soil; WS: Q. wutaishanica rhizosphere soil

The Shannon and Simpson diversity indices consistently showed certain differences in the alpha diversities of rhizosphere bacterial communities (*Table 1*), and there were significant differences between AS and VS, showing that of AS was the highest and VS the lowest. Thus, AS and VS had the highest and lowest bacterial alpha diversity in roots, respectively. In addition, the species richness of bacteria in different groups of soil samples was also different, and the ACE and Chao indices of AS were significantly higher than those of WS.

### *Beta diversity*

The Bray–Curtis NMDS results are shown in *Figure 2*. Overall, the soil bacterial communities of different *Quercus* species clustered into different groups, indicating that the bacterial community structures in different *Quercus* species soils were different.

DOI: http://dx.doi.org/10.15666/aeer/2303_52395251

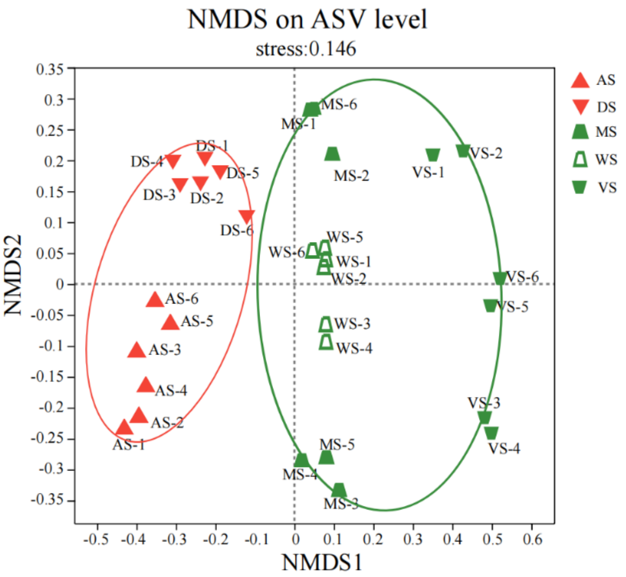

***Figure 2.*** *Non-metric multidimensional scaling (NMDS) analysis of rhizosphere bacteria of five different Quercus species. AS: Q. aliena rhizosphere soil; DS: Q. dentate rhizosphere soil; MS: Q. mongolica rhizosphere soil; VS: Q. variabilis rhizosphere soil; WS: Q. wutaishanica rhizosphere soil*

However, the rhizosphere soil samples from the five *Quercus* spp. were separated into two groups based on NMDS1: the rhizosphere bacterial communities from AS and DS were very similar to the first group (red ellipse in *Figure 2*), and MS, WS, and VS were very similar to the second group (green ellipse in *Figure 2*). Interestingly, we found that the beta diversity of the rhizosphere bacterial communities was consistent with the genetic relationships of *Quercus*.

### *Rhizosphere soil bacterial communities*

Changes in soil bacterial composition caused by different *Quercus* species were mainly caused by changes in the relative abundance of several bacteria (*Figure 3*). The two dominant phyla with obvious changes in relative abundance were Proteobacteria (28.7%–38.9%) and Firmicutes (11.2%–23.9%). In addition, the relative abundances of Bacteroidota (4.79%) and Acidobacteria (14.5%) were highest in VS, while the relative abundance of Bdellovibeionota (0.53%) was significantly lower in VS. In addition, the relative abundance of Proteobacteria, Bacteroidota, Bdellovibeionota, Gemmatimonadota, and Acidobacteria did not differ significantly between AS and DS, while the relative abundances of Bacteroidota, Patescibacteria, and *Bryobacter* in MS were similar to those in WS.

### *Function analysis*

Based on the KEGG pathway database, bacterial functions were predicted using PICRUSt2. The nine bacterial functions that changed significantly are shown in *Figure 4*. Amino acid metabolism (10.43%–10.74%) was the main metabolic pathway in the rhizosphere bacterial communities of the five *Quercus* species, followed by transcription (5.67%–6.25%) and carbohydrate metabolism (5.60%–6.29%). This indicated that amino acid metabolism is the major carbon (C), nitrogen (N), and energy source utilized in bacterial metabolism. In addition, the relative abundance of amino acid metabolism, transcription, and carbohydrate metabolism was highest in AS, followed by DS, and lowest in MS. This indicated that the major metabolic functions of bacteria in AS and DS were higher than those in MS, WS, and VS.

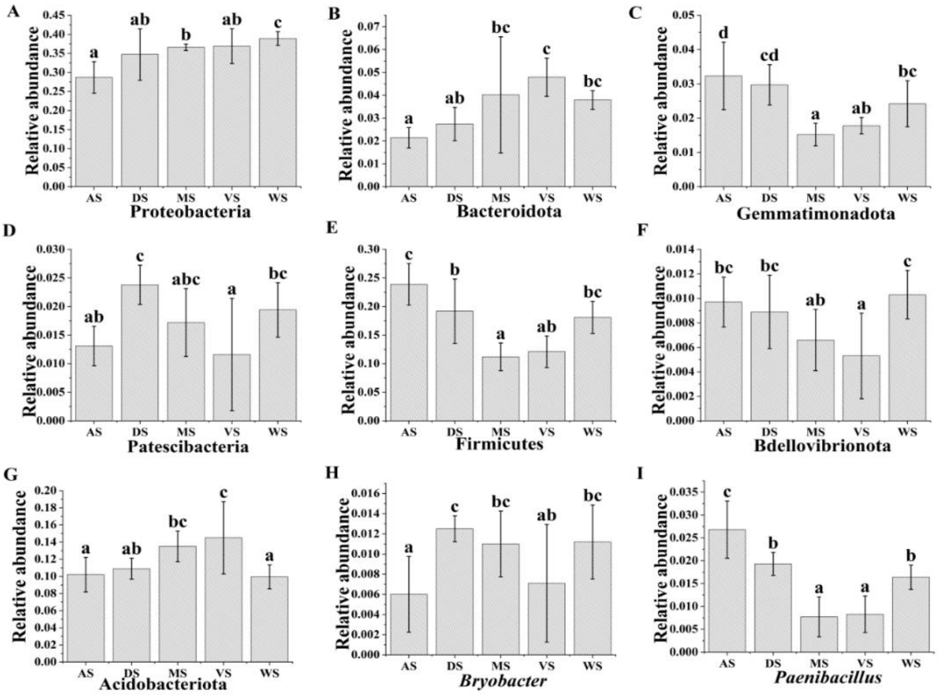

**Figure 3.** *Relative abundance of two bacterial phyla (A–G) and genera (H–I) in the soil affected by different Quercus species. Within each panel, uppercase letters indicate significant differences at P < 0.05 across patch type. AS: Q. aliena rhizosphere soil; DS: Q. dentate rhizosphere soil; MS: Q. mongolica rhizosphere soil; VS: Q. variabilis rhizosphere soil; WS: Q. wutaishanica rhizosphere soil*

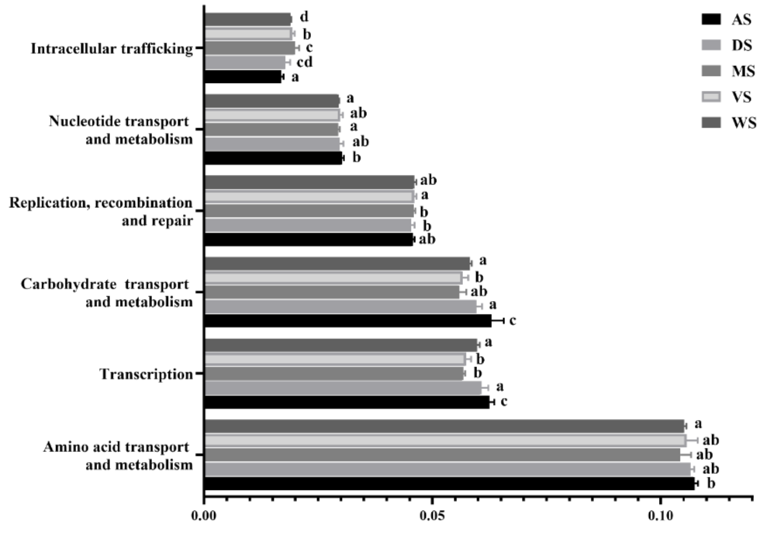

**Figure 4.** *The relative abundance of Kyoto Encyclopedia of Genes and Genomes (KEGG)-assigned functional categories in the groups. Within each panel, uppercase letters indicate significant differences at P < 0.05 across patch type*

## Discussion

We used rhizosphere soil differences among five *Quercus* species to assess how plant genotypes influence the communities and abundance of bacterial communities in the rhizosphere. Our results showed that rhizosphere bacterial communities were strongly influenced by plant genotype.

### *The role of genetic effects in shaping rhizosphere bacterial communities*

Previous studies have shown that the plant genotype is an important biological factor influencing the composition of rhizosphere microorganisms, which can affect the composition and abundance of bacterial and fungal communities by producing different hormones and secretions (Wagner et al., 2016; Dhungana et al., 2023; Ramirez-Villacis et al., 2023). In our study, the NMDS results also showed that the rhizosphere bacterial communities of different *Quercus* species could be divided into two major groups: AS and DS were very similar and formed the first group, and MS, WS, and VS were the second group. The results showed that plant genotype was the decisive factor for differences in the rhizosphere microbial communities of different *Quercus* species. In addition, the relationship between the rhizosphere bacterial communities of the five *Quercus* species shown using the NMDS results was consistent with the four genetic clusters of *Quercus* species shown in the phylogenetic tree. This further verified the effect of the genotype of *Quercus* spp on the rhizosphere bacteria of different *Quercus* species.

Statistical analysis revealed a significant difference in the OTU richness and bacterial diversity of different *Quercus* species ($P < 0.05$), indicating that the genetic relationship between plants affects the richness and diversity of rhizosphere bacteria. This is consistent with the findings of previous studies (Wagner et al., 2016). *Quercus* sp. rhizosphere bacteria were mainly composed of Proteobacteria, Firmicutes, and Acidobacteria. These findings were expected because Proteobacteria are well-adapted to the rhizospheres of different plant species (Yang et al., 2023), and Acidobacteria are one of the most abundant bacterial taxa in soils, especially under acidic conditions (Lee et al., 2008). Additionally, Proteobacteria and Acidobacteria were the most dominant bacterial phyla in the rhizosphere of other *Quercus* species (Yan et al., 2020; Wang et al., 2023). However, there were significant differences in the relative abundances of Proteobacteria, Firmicutes, Acidobacteria, and other bacteria among the five *Quercus* species (*Figure 2*). These results indicated that the *Quercus* spp. genotype mainly affects the relative abundance of rhizosphere bacteria, which is consistent with a previous study (Cheng et al., 2020). Moreover, since the genetic relationship between *Q. Aliena* and *Q. dentate* is relatively close, the relative abundance of rhizosphere bacteria (such as Proteobacteria, Bacteroidota, Bdellovibeionota, Gemmatimonadota, and Acidobacteria) was similar, whereas the relative abundance of rhizosphere bacteria (such as Bacteroidota and Acidobacteria) varied greatly due to the genetic relationship between *Q. variabilis* and the other relatively distant species. This suggests that the relative abundance of rhizosphere bacteria is correlated with the genetic relationship between different *Quercus* species.

Studies have shown that root exudates from different plant species can affect the growth of rhizosphere bacteria by altering the physicochemical properties of the soil (Mommer et al., 2016; Dhungana et al., 2023). For example, the plant-available concentrations of ions, such as phosphorus (P) and soil organic carbon (SOC), increase with the exudation of acid phosphatases, protons, and carboxylates (Duffner et al., 2012;

Delgado-Baquerizo et al., 2017; Li et al., 2023). In addition, a previous study claimed that the concentration of P strongly affected the microorganisms involved in P metabolism and, thus, the relative abundance of rhizosphere bacteria, especially Firmicutes and Proteobacteria (Ma et al., 2023). Additionally, increasing the P content in the soil can increase the soil pH, which indirectly affects relative abundance of rhizosphere bacteria, such as Acidobacteria (Huang et al., 2016; Kim et al., 2021). Thus, we hypothesized that the root exudates of different *Quercus* species were different, which altered the physicochemical properties of the soil, such as P and pH, and affected the relative abundance of rhizosphere bacteria, such as Proteobacteria, Firmicutes, and Acidobacteria; however, further validation is required.

Besides, *Quercus* spp. use their fine roots to colonise ectomycorrhiza fungi and form mycorrhiza circles (the area of soil affected by mycorrhiza) and mycelium circles (the area of soil affected by mycorrhiza) (Prescott and Grayston, 2013). Due to some ectomycorrhizal fungal communities have high host specificity, ectomycorrhizal fungi can also affect the community structure of rhizosphere bacteria (Buée et al., 2011). Furthermore, Studies have shown that many forest trees have evolved symbiotic relationships with ectomycorrhizal fungi that facilitate their phosphorus nutrient uptake, translocation, and transfer to plant hosts (Cairney, 2011). This may also be one of the reasons for affecting the rhizosphere bacteria of different *Quercus* species. Therefore, in future research, we should further investigate the relationships among ectomycorrhizal fungi and rhizosphere bacteria of different *Quercus* species.

However, the phenotypic traits of different genotypes of plants differ, and the long-term accumulation of plant phenotypic traits in the rhizosphere leads to different physicochemical characteristics of the soil in the root zone (Li et al., 2018), thus affecting the microbial community in the rhizosphere, especially with respect to the active bacterial populations (Ofek et al., 2013). Plant litter alters soil bacterial communities by affecting the amount of dissolved organic carbon and total nitrogen in the soil (Madritch and Lindroth, 2011; Wang et al., 2022a). Moreover, the morphology and microbial community of the leaves and acorns of *Quercus* spp. are affected by genetic factors (Wagner et al., 2016; Cavender-Bares, 2018); that is, the litter of different *Quercus* spp. is different. However, since this experiment only lasted for six months, the effects of litter may be small.

### *Effects of genetic relationships on the function of bacterial community*

Differences in the structure and composition of bacterial communities often lead to changes in their functions, such as metabolic capacity and biodegradation (Zhao et al., 2024). In our study, amino acid and carbohydrate metabolisms were the major metabolic pathways involved, particularly in AS and DS. The probable reason for this is that there are more rhizosphere bacteria in AS and DS that metabolize amino acids and carbohydrates. For example, Firmicutes can degrade proteins, carbohydrates, and amino acids (Yang and Song, 2019), and the capability of carbohydrate metabolism in *Paenibacillus* is high (Ribeiro et al., 2022). In addition, the relative abundance of Firmicutes and *Paenibacillus* was highest in AS, followed by DS. Moreover, studies have shown that the C cycle in the soil also affects the metabolic pathways of bacteria (Li et al., 2020), indicating that the physicochemical properties of soil are some of the factors that cannot be ignored.

In addition, we suppose that the more likely reason for this is that rhizosphere exudates vary across *Quercus* spp., which affects the metabolic levels of rhizosphere bacteria.

Previous studies have shown that the most abundant compound groups in the root exudates of *Quercus* species are amino acids, carbohydrates, and organic acids (Preece et al., 2022) and that plant species affect the composition of their root exudates (Dhungana et al., 2023). Thus, we suppose that the root exudates of different *Quercus* species may have different amino acid, carbohydrate, and organic acid contents, leading to different bacterial metabolic pathways. In conclusion, the relationship between rhizosphere exudates of different genotypes of *Quercus* spp. deserves further investigation.

## Conclusions

We found that genetic relationships were important factors leading to differences in the rhizosphere bacterial communities of different *Quercus* spp. The relative abundances of rhizosphere bacteria in *Quercus* spp. with close genetic relationships were similar. In contrast, the relative abundances of rhizosphere bacteria in the genus *Quercus* with distant genetic relationships were significantly different. In addition, based on previous studies, we speculated that differences in rhizosphere exudates and phenotypic characteristics of different *Quercus* spp. influenced the relative abundance and function of rhizosphere bacteria. However, the relationship between these factors and the rhizosphere bacteria of *Quercus* requires further study. In addition, tests of soil physicochemical properties and the composition and content of root exudates in different *Quercus* spp. should be used for further validation.

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

**Acknowledgments.** This work was partially supported by the National Natural Science Foundation of China [No. 32170530].

**Conflict of interest.** The authors declare that they have no conflict of interest.

**Data availability.** 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 PRJNA1047939.

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