Postprint: Soil Microbial Community Structure in Rhizosphere and Non-rhizosphere of Typical Forest Stands in the Qinling Mountains During Summer and Autumn

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Abstract

This study primarily focused on four typical forest stands in the Qinling Mountains—Quercus aliena var. acutidentata, Pinus tabuliformis, Pinus armandii, and Picea asperata—and utilized the BIOLOG microplate method to investigate the metabolic diversity of rhizosphere and bulk soil microbial communities during summer and autumn. The results demonstrated that: (1) The average well color development (AWCD) values of rhizosphere and bulk soils differed distinctly between summer and autumn. Except for Picea asperata in autumn, all other treatments exhibited bulk soil > rhizosphere, and AWCD was significantly influenced by season. (2) Significant differences existed in soil microbial functional diversity between rhizosphere and bulk soils across the four forest stands in both seasons. Specifically, Quercus aliena var. acutidentata forest consistently showed rhizosphere < bulk soil in both summer and autumn, whereas the other three coniferous forests displayed rhizosphere < bulk soil in summer but rhizosphere > bulk soil in autumn. For Quercus aliena var. acutidentata forest, both rhizosphere and bulk soils were lower in autumn than in summer; conversely, for the other three coniferous forests, bulk soil was lower in autumn than in summer, while rhizosphere soil was higher in autumn than in summer. (3) Principal component analysis revealed significant differences in soil microbial functional diversity, and the composite scores of principal components for rhizosphere and bulk soils of the four forest stands also varied between summer and autumn. Among bulk soils, Quercus aliena var. acutidentata exhibited the highest composite score, followed by Pinus armandii and Picea asperata, with Pinus tabuliformis being the lowest. The pattern for rhizosphere soil differed between seasons: in summer, Pinus armandii and Pinus tabuliformis were higher while Picea asperata was lowest; in autumn, Pinus tabuliformis and Picea asperata were higher while Quercus aliena var. acutidentata was lowest. Moreover, composite scores showed significant or highly significant positive correlations with diversity indices. (4) Redundancy analysis indicated that the combined effects of soil physicochemical properties significantly influenced soil microbial community functional diversity.

Full Text

Preamble

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Characteristics of Microbial Community in Forest Soil Between Rhizosphere and Non-rhizosphere in Summer and Autumn in Qinling Mountains, China

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Abstract

This study investigated the functional diversity of microbial communities in rhizosphere and non-rhizosphere soils during summer and autumn across four typical forest types in the Qinling Mountains: *Quercus aliena* var. *acutidentata* (tooth oak), *Pinus tabuliformis* (Chinese pine), *Pinus armandii* (Armand pine), and *Picea asperata* (spruce), using the micro-BIOLOG method. The results showed that: (1) The Average Well Color Development (AWCD) values differed significantly between rhizosphere and non-rhizosphere soils in most cases, with non-rhizosphere soils showing higher AWCD than rhizosphere soils, except for spruce in autumn. (2) Microbial functional diversity differed remarkably between rhizosphere and non-rhizosphere soils for all four forest types in both seasons. For tooth oak, rhizosphere diversity was lower than non-rhizosphere diversity in both summer and autumn. For the three coniferous species, this pattern was observed in summer, but the trend reversed in autumn. Additionally, diversity in autumn was lower than in summer for non-rhizosphere soils, but higher for rhizosphere soils. (3) Principal component analysis revealed significant differences in microbial community functional diversity among the four forest types, with principal component scores varying substantially between rhizosphere and non-rhizosphere soils across seasons. The highest scores were found in non-rhizosphere soils under tooth oak, followed by Armand pine and spruce, with Chinese pine showing the lowest scores. Rhizosphere soil scores showed seasonal variation: Chinese pine and Armand pine had relatively high scores in summer, while Chinese pine and spruce had high scores in autumn. The comprehensive scores were positively or extremely positively correlated with diversity indices. (4) Redundancy analysis demonstrated that the combined effects of soil physicochemical properties significantly influenced microbial community functional diversity.

Keywords: soil microorganisms; rhizosphere; non-rhizosphere; diversity index; season

Introduction

The Qinling Mountains serve as China' s primary north-south climate boundary and play crucial roles in climate regulation, carbon sequestration, and protection of rare species. Their unique geographic location and complex natural environment have fostered rich plant resources, with typical vegetation types distributed across different elevation ranges, including tooth oak, Chinese pine, Armand pine, and spruce [1]. The rhizosphere is one of the most active sites for plant energy and material metabolism, containing far more microorganisms than bulk soil, and rhizosphere microbes respond to nutrients released by roots [2-3]. Their physiological activities significantly affect soil properties and plant nutrient uptake, ultimately influencing plant growth [4-6] and leading to changes in plant community structure and terrestrial ecosystem carbon cycling patterns. Rhizosphere microbes are important for improving soil fertility and maintaining soil ecological environments [7].

Previous studies using molecular biological methods have shown that forest type affects soil microbial communities [8] and exhibits obvious seasonal variation characteristics [9]. Significant differences exist among tree species in microbial community diversity and preferential carbon source utilization in mycorrhizal rhizospheres [10-11]. Natural secondary forests have higher soil microbial community diversity than plantations and wastelands, revealing that natural restoration is more conducive to improving soil microbial structure and function [12]. In the Qinling region, rhizosphere microbial numbers in larch forests are greater than in pine-oak mixed forests [14]. Bacteria and fungi in soil have long been research hotspots and challenges in soil ecology [13, 15]. These findings indicate that forest ecosystem restoration and protection must consider not only plant diversity but also soil microbial diversity. However, systematic studies on how typical forest types influence rhizosphere and non-rhizosphere soil microbial characteristics in the Qinling region are lacking [16]. Therefore, it is necessary to investigate soil microbial characteristics in the Qinling Mountains to reveal seasonal variation patterns in forest ecosystem attributes and provide scientific basis for the protection and sustainable development of coniferous and broadleaf forest ecosystems in this region.

This study examined four typical forest types in the Qinling Mountains, investigating seasonal changes in functional diversity of rhizosphere and non-rhizosphere soil microbial communities during summer and autumn, identifying dominant carbon source types and metabolic characteristics, and analyzing the relationships between microbial communities and environmental factors to provide a basis for directed regulation of forest development and ecosystem protection.

1. Experimental Materials

Study sites were selected in the Qinling Mountains, including tooth oak and Armand pine plots at Matoutan Forest Farm, and Chinese pine and spruce plots at Xinjialin Forest Farm. Three standard 20 m × 20 m plots were established in each forest type. Within each plot, three trees of similar age were selected. Rhizosphere soil samples were collected from the 0–10 cm soil profile within 2 mm of the root surface using sterile methods in both summer and autumn. Non-rhizosphere soil samples were collected from the same depth. After removing surface litter, five sampling points were established in each plot and soil samples were mixed. Fresh soil samples were stored at 4°C for microbial diversity analysis, while remaining soils were air-dried for physicochemical analysis.

2. Experimental Methods

Air-dried soil samples were analyzed for basic physicochemical properties including pH, organic matter, total nitrogen, total phosphorus, total potassium, available nitrogen, available phosphorus, available potassium, and slowly available potassium using conventional methods [17]. Fresh soil samples (10 g) were suspended in 45 mL of 0.85% NaCl solution, shaken at 200 r/min for 30 minutes, and serially diluted. The suspension (150 L) was inoculated into each well of BIOLOG EcoTest plates and incubated at (25±1)°C. Absorbance was measured at 590 nm after 120 hours. The 31 sole carbon sources were divided into six categories [18]: carbohydrates, carboxylic acids, amino acids, amines, polymers, and aromatic compounds.

3. Data Analysis

Average Well Color Development (AWCD): Calculated as the average absorbance of all carbon source wells. Wells with absorbance values less than zero were recorded as zero in calculations.

Gompertz Equation Fitting: The relationship between AWCD and incubation time was fitted using the Gompertz equation [20]:

$$OD_{590} = A \times \exp[-\exp(e/A \times (U_m \times t) + 1)]$$

where A is maximum absorbance, U_m is maximum absorbance change rate, and t is incubation time.

Diversity Indices: Community richness index, Shannon diversity index (H'), McIntosh diversity index, and Gini index were calculated. A well with AWCD > 0.2 was considered positive, representing utilized carbon source.

Statistical Analysis: Data were processed using Microsoft Excel 2003 and SPSS 12.0. One-way ANOVA was performed on microbial diversity indices and carbon source utilization. Principal component analysis (PCA) and redundancy analysis (RDA) were conducted using CANOCO 4.5 to analyze relationships between soil physicochemical properties and carbon source utilization patterns.

2. Results

2.1 Changes in Soil Microbial Average Well Color Development (AWCD)

AWCD reflects microbial community carbon source utilization rate and is an important indicator of microbial community functional diversity. During 24-120 hours of incubation, AWCD increased over time, indicating carbon source utilization. At the same incubation time, AWCD values differed significantly between rhizosphere and non-rhizosphere soils across seasons. Except for spruce in autumn, all other treatments showed higher AWCD in non-rhizosphere than rhizosphere soils. Seasonal effects were evident: for tooth oak and Armand pine, non-rhizosphere soils showed faster utilization in summer, while other treatments showed the opposite pattern.

Gompertz equation fitting yielded R^2 values > 0.974, indicating good representation of the data. The maximum absorbance change rate (U_m) revealed the fastest carbon source utilization rate by microbes. Most treatments showed faster utilization rates in non-rhizosphere soils, except for Chinese pine in summer and spruce in autumn. Seasonal patterns varied by forest type. Maximum absorbance (A) reflected overall carbon source utilization capacity, with most treatments showing greater capacity in non-rhizosphere soils, except for Chinese pine and spruce in autumn where rhizosphere soils were higher. The time required to reach exponential growth phase (t) was generally shorter in non-rhizosphere soils, with seasonal variations observed across different forest types.

[Figure 1: see original paper] AWCD of four forest types. TO, CP, AP, SP represent tooth oak, Chinese pine, Armand pine, and spruce, respectively. -N indicates non-rhizosphere soil; -R indicates rhizosphere soil.

2.2 Changes in Soil Microbial Community Diversity Indices

Microbial community functional diversity reflects the ecological characteristics of soil microbes. Significant differences in diversity indices were observed among forest types and between rhizosphere and non-rhizosphere soils. For tooth oak, all diversity indices were higher in non-rhizosphere than rhizosphere soils in both seasons, with values decreasing from summer to autumn in non-rhizosphere soils but increasing in rhizosphere soils. For the three coniferous species, diversity indices were higher in non-rhizosphere than rhizosphere soils in summer, but this pattern reversed in autumn. Seasonal changes showed that non-rhizosphere soils had lower diversity in autumn than summer, while rhizosphere soils showed the opposite trend.

Soil microbial community diversity indices. Values are means ± SE. Different letters within the same column indicate significant differences ($P < 0.05$).

2.3 Carbon Source Utilization by Soil Microbes

Microbial utilization of different carbon sources reflects metabolic functional groups. The utilization patterns of six carbon source categories were generally consistent with diversity indices. Dominant carbon sources varied by forest type: tooth oak primarily utilized amino acids and polymers; Chinese pine utilized amino acids and amines; Armand pine and spruce utilized carbohydrates and amino acids. Weakly utilized carbon sources were primarily carboxylic acids and aromatic compounds across all forest types. However, dominant and weak carbon sources shifted seasonally within each forest type.

Parameters of Gompertz equation fitting.

2.4 Principal Component Analysis of Microbial Community Function

To identify the main factors influencing soil microbial characteristics under different forest types, principal component analysis was performed on absorbance values of 31 carbon sources. The first two principal components explained 76.97%-95.58% of the variance, indicating clear differentiation among treatments. The analysis distinguished rhizosphere from non-rhizosphere soils along PC1 and separated summer from autumn samples along PC2. Tooth oak and Chinese pine showed similar patterns, while Armand pine and spruce exhibited comparable trends.

[Figure 2: see original paper] Principal component analysis of soil bacterial communities for different tree species.

Further analysis of principal component scores revealed that dominant carbon sources differed among forest types. Along PC1, tooth oak showed high utilization of putrescine and -lactone; Chinese pine utilized primarily -lactose and methyl pyruvate; Armand pine utilized cyclodextrin and -lactose; spruce utilized phenylalanine and phenylethylamine. PC2 showed seasonal patterns in carbon source utilization.

2.5 Redundancy Analysis

Comprehensive principal component scores varied significantly among forest types and seasons. For tooth oak, non-rhizosphere scores were higher than rhizosphere scores. For Armand pine and spruce, non-rhizosphere scores were higher in summer but lower in autumn. Correlation analysis revealed significant or extremely significant positive correlations between comprehensive scores and diversity indices.

Redundancy analysis further identified the effects of soil physicochemical properties on microbial carbon source utilization patterns. Soil properties significantly influenced microbial community functional diversity, but the specific environmental factors varied by forest type. For tooth oak, organic matter, available phosphorus, and C:N ratio were positively correlated with carbon source utilization. For Chinese pine, available potassium and C:N ratio showed positive correlations. For Armand pine, available phosphorus and available potassium were positively correlated with most carbon sources. For spruce, only C:N ratio and available phosphorus showed positive correlations.

[Figure 3: see original paper] Redundancy analysis of 31 sole carbon sources and environmental variables. Solid arrows represent carbon sources; dashed arrows represent environmental factors.

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3. Discussion

This study revealed substantial differences in microbial functional diversity between rhizosphere and non-rhizosphere soils, with seasonal changes affecting these compartments differently. In summer, all four forest types showed lower microbial functional diversity indices in rhizosphere than non-rhizosphere soils. This is primarily because the rainy season concentrates in summer, and high temperature and moisture create favorable conditions for microbial growth and reproduction. However, under certain conditions, the selective effect of root exudates can also lead to lower functional diversity in rhizosphere soils, known as the rhizosphere negative effect. Additionally, phenolic substances and vanillin in Chinese fir root exudates can cause autotoxicity [21], and oak root exudates can inhibit nitrogen-fixing bacteria [22-23], both reducing rhizosphere microbial numbers below non-rhizosphere levels. Furthermore, herbaceous and shrub densities in summer forest areas are much higher than tree densities, and these plants also influence soil microbial communities.

In autumn, tooth oak rhizosphere soils showed lower microbial functional diversity than non-rhizosphere soils, but other forest types showed the opposite pattern. This difference is mainly attributed to forest type characteristics. Tooth oak has fewer fibrous roots in the 0–10 cm surface soil layer, and dense understory shrubs in oak forests limit root influence on soil microbes. In contrast, Armand pine and spruce have more developed fibrous root systems in the surface soil, producing greater quantities and varieties of root exudates and detritus. Well-developed fibrous roots also protect rhizosphere soils, reducing the impact of external environmental changes on microbial functional diversity. Sugden [24] found that higher soil microbial diversity indices correspond to stronger ecosystem resistance to external disturbances. Compared with tooth oak, the three coniferous forests have fewer shrubs, mainly herbaceous plants that senesce in autumn, reducing their influence on soil microbes.

Different forest types showed significant differences in utilization of six carbon source categories. Tooth oak communities primarily metabolized amino acids and polymers; Chinese pine communities metabolized amino acids and amines; Armand pine and spruce communities metabolized carbohydrates and amino acids. Weakly utilized carbon sources were mainly carboxylic acids and aromatic compounds across all forest types. These differences are related to tree density, shrub density, herb density, and litter fall amounts (Table 1), corroborating that microbial community structure and function are associated with the quantity and biochemical composition of litter from different dominant tree species and are closely related to root exudates [25-26]. Root exudates can improve rhizosphere nutrient environments and enhance nutrient bioavailability, significantly influencing the quantity and activity of soil microbes around tree roots [14, 27].

Redundancy analysis revealed significant effects of soil physicochemical properties on microbial carbon source utilization. In rhizosphere and non-rhizosphere soils across both seasons, soil alkaline hydrolyzable nitrogen and available phosphorus were closely related to microbial carbon utilization. This is because forest soils have much higher carbon content than nitrogen content, with low alkaline hydrolyzable nitrogen and very low available phosphorus, making these nutrients limiting factors for microbial growth and reproduction. Soil pH was negatively correlated with carbon source utilization for most carbon sources, possibly because acidic forest soil conditions inhibit bacterial populations from utilizing carbon sources [28].

Basic information of sample plots.

4. Conclusion

This study demonstrated that: (1) Across the four forest types, microbial functional diversity in tooth oak rhizosphere soils was lower than in non-rhizosphere soils in both summer and autumn, while for coniferous forests, rhizosphere diversity was lower in summer but higher in autumn compared to non-rhizosphere

soils. (2) Seasonal differences in microbial functional diversity were significant: non-rhizosphere soils showed lower diversity in autumn than summer, while rhizosphere soils showed the opposite trend. (3) Principal component analysis revealed distinct differences in comprehensive scores between rhizosphere and non-rhizosphere soils across seasons, with significant or extremely significant positive correlations between comprehensive scores and diversity indices. Among non-rhizosphere soils, tooth oak had the highest comprehensive score, followed by Armand pine and spruce, with Chinese pine the lowest. In rhizosphere soils, Chinese pine and Armand pine had higher scores in summer, while Chinese pine and spruce had higher scores in autumn. (4) Redundancy analysis indicated that the combined effects of soil physicochemical properties significantly influenced microbial community functional diversity, with different key factors for each forest type.

References

[1] Study on niche characteristics of *Quercus aliena* var. *acutidentata* populations in Qinling Mountains. *Chinese Journal of Applied Ecology*, 2004, 15(6): 913-918.

[2] Marilley L, Vogt G, Blanc M, Aragno M. Bacterial diversity in the bulk soil and rhizosphere fractions of *Lolium perenne* and *Trifolium repens* revealed by PCR restriction analysis of 16S rDNA. *Plant and Soil*, 1998, 198(2): 219-224.

[3] Jjemba PK, Alexander M. Possible determinants of rhizosphere competence of bacteria. *Soil Biology and Biochemistry*, 1999, 31(4): 623-632.

[4] Effects of different varieties and farming methods on rhizosphere microorganisms of sweet potato in saline-alkali soils.

[5] Isolation and identification of *Bacillus globisporus*.

[6] Preliminary study on rhizosphere microorganisms of tobacco under different fertility conditions.

[7] Research progress on influencing factors of plant rhizosphere microorganisms. *Henan Agricultural Sciences*, 2014, 43(5): 6-12.

[8] Grayston SJ, Wang S, Campbell CD, Edwards AC. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry*, 1998, 30(3): 369-378.

[9] Analysis of soil microbial population quantity and nutrient changes under different forest types. *Sichuan Forestry Science and Technology*, 1997, 18(4): 32-35.

[10] Characteristics of rhizosphere soil microbial community structure and function of typical forest trees in the Loess Plateau and their environmental significance. *Chinese Journal of Applied Ecology*, 2009, 30(8): 2432-2437.

[11] Study on microbial community diversity in mycorrhizal rhizosphere soils of three afforestation tree species. *Journal of Beijing Forestry University*, 2008, 30(3): 85-90.

[12] Effects of different forest restoration types on soil microbial communities. *Chinese Journal of Applied Ecology*, 2004, 15(11): 2019-2024.

[13] Zhang B, Yang YS, Zepp H. Effect of vegetation restoration on soil and water erosion and nutrient losses of a severely eroded clayey Plinthudult in southeastern China. *Catena*, 2004, 57(1): 77-90.

[14] Study on rhizosphere microorganisms and soil biochemical characteristics in Huoditang Forest Region of Qinling Mountains.

[15] Characteristics of soil microbes in several typical forests of Qinling Mountains and their response to human disturbance. *Journal of Northwest Forestry University*, 2008, 23(2): 129-134.

[16] Cheng F, Peng XB, Zhao P, Yuan J, Zhong CG, Cheng YL, Cui C, Zhang SX. Soil microbial biomass, basal respiration and enzyme activity of main forest types in the Qinling Mountains. *PLoS One*, 2013, 8(6): e67353.

[17] *Soil Agrochemical Analysis*. China Agricultural Press, 2000.

[18] Papatheodorou EM, Efthimiadou E, Stamou GP. Functional diversity of soil bacteria as affected by management practices and phenological stage of *Phaseolus vulgaris*. *European Journal of Soil Biology*, 2008, 44(4): 429-536.

[19] Kong WD, Zhu YG, Fu BJ, Marschner P, He JZ. The veterinary antibiotic oxytetracycline and Cu influence functional diversity of the soil microbial community. *Environmental Pollution*, 2006, 143(1): 129-137.

[20] Verschuere L, Fievez V, Van Vooren L, Verstraete W. The contribution of individual populations to the BIOLOG pattern of model microbial communities. *FEMS Microbiology Ecology*, 1997, 24(4): 353-362.

[21] Huang ZQ, Liao LP, Wang SL, Cao GQ. Allelopathy of phenolics from decomposing stump-roots in replant Chinese fir woodland. *Journal of Chemical Ecology*, 2000, 26(9): 2211-2219.

[22] Preliminary study on rhizosphere soil microbes and enzyme activities in Chinese fir. *Forest Research*, 1993, 25(5): 271-273.

[23] Study on rhizosphere soil microbes and biochemical characteristics of natural and planted *Castanopsis kawakamii* forests. *Acta Ecologica Sinica*, 1998, 18(2): 198-202.

[24] Sugden AM. Diversity & ecosystem resilience. *Science*, 2000, 290(5490): 233-235.

[25] Johansson MB. The chemical composition of needle and leaf litter from Scots pine, Norway spruce and white birch in Scandinavian forests. *Forestry*, 1995, 68(1): 49-62.

[26] Grayston SJ, Campbell CD. Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*). *Tree Physiology*, 1996, 16(11-12): 1031-1038.

[27] Changes in quantity and types of rhizosphere and non-rhizosphere soil microbes in different generations of Chinese fir plantations. *Forest Research*, 1999, 12(1): 13-18.

[28] Functional diversity of soil microbes under typical artificial forests in the hilly and gully region of Loess Plateau. *Acta Ecologica Sinica*, 2009, 29(2): 727-733.

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