

Postprint: Functional Diversity of Rhizosphere and Non-rhizosphere Soil Microorganisms of Dominant Herbaceous Plants in Dushanzi District

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Abstract

Taking the rhizosphere and non-rhizosphere soils of three dominant herbaceous plants in the Dushanzi District as the research object, soil microbial functional diversity was investigated using the Biolog-ECO microplate method. The results indicated that: the microbial metabolic activity (AWCD), Shannon richness index (H), and McIntosh evenness index (U) of rhizosphere and non-rhizosphere soils among the three plant species all showed varying degrees of difference, with the microbial functional diversity in the rhizosphere soil of *Artemisia borotalensis* being superior to that in non-rhizosphere soil and the other two plants; rhizosphere soil microorganisms exhibited relatively high sensitivity to carbohydrate, lipid, acid, and amine carbon sources, whereas non-rhizosphere soil microorganisms were sensitive to acid, amino acid, and carbohydrate carbon sources; rhizosphere soil microorganisms possessed stronger carbon source utilization capacity, and the carbon source utilization characteristics of microorganisms varied among the rhizosphere environments of different plants; microbial activity, richness index, and microbial evenness index demonstrated significant positive correlations with soil pH, SOM, AP, and NO₃-N ($P < 0.05$); the rhizosphere soil of *Artemisia borotalensis* exhibited higher nutrient content and microbial activity, along with stronger environmental adaptability, thus warranting attention in ecological environment management and construction in the Dushanzi District.

Full Text

Preamble

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Soil Microbial Functional Diversity of Rhizosphere and Non-Rhizosphere of Three Dominant Herbaceous Plants in the Dushanzi District

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Abstract

This study investigated the soil microbial functional diversity in rhizosphere and non-rhizosphere soils of three dominant herbaceous plants in the Dushanzi District using the Biolog-ECO microplate method. The results showed that microbial metabolic activity, richness index, and evenness index differed significantly between rhizosphere and non-rhizosphere soils across the three plant species. *Artemisia borotalensis* rhizosphere soil exhibited superior microbial functional diversity compared to its non-rhizosphere soil and to the rhizosphere soils of the other two species. Rhizosphere soil microbes were particularly sensitive to carbohydrate, lipid, acid, and amine carbon sources, whereas non-rhizosphere soil microbes showed sensitivity primarily to acids, amino acids, and carbohydrates. Rhizosphere soil microorganisms demonstrated stronger carbon source utilization capacity, with distinct carbon utilization profiles characterizing different plant rhizosphere environments. Significant positive correlations ($p < 0.05$) were observed between microbial richness and evenness indices and soil pH, soil organic matter (SOM), and available phosphorus (AP) in the Dushanzi ecological environment. The *Artemisia borotalensis* rhizosphere soil showed higher nutrient content and microbial activity, indicating stronger environmental adaptability and suggesting its potential value for ecological management and restoration efforts in the Dushanzi District.

Keywords: dominant species; rhizosphere soil; microbial functional diversity; Dushanzi District; Biolog-ECO

Introduction

Soil microorganisms serve as the engines of biogeochemical cycling and play crucial roles in maintaining ecosystem services and terrestrial ecosystem stability. The diversity of all microbial species, genes, and their interactions with the environment constitutes soil microbial diversity. Functional diversity represents a key indicator of soil microbial community status and function, encompassing

nutrient cycling and functions that either promote or inhibit plant growth. Understanding microbial functional diversity is essential for clarifying the role of microbial communities in different environments and for assessing soil ecological functions.

Soil microbial diversity is directly influenced by environmental conditions such as soil microdomain structure and spatial heterogeneity. The rhizosphere, as one of the primary microdomain types, has been extensively studied in various contexts, including saline-alkali soils, fertilized soils, and contaminated soils—most of which represent human-disturbed, non-natural ecosystems. Previous research on natural ecosystems such as grasslands has revealed that rhizosphere soil microbial characteristics vary with region, land use patterns, and growth duration. However, studies on soil microbial functional diversity in arid regions remain limited.

The Dushanzi District of Karamay City, Xinjiang, serves as a major petrochemical base and strategic hub for oil and gas import, storage, and transfer in western China, as well as a key development area on the northern slope of the Tianshan Mountains. With nearly a century of oil extraction and processing, soil problems related to industrial activities have become increasingly prominent, making ecological restoration an urgent priority. Successful ecosystem restoration requires attention not only to plant diversity but also to soil microbial diversity, which is vital for soil structure formation, aggregate stability, and organic matter transformation. Vegetation provides nutrients and energy for soil microorganisms and influences microbial diversity through its effects on soil organic carbon, nitrogen, phosphorus levels, and pH.

This study examined rhizosphere and non-rhizosphere soils of three dominant herbaceous plants in the Dushanzi District to investigate soil chemical properties, microbial average well color development (AWCD), microbial diversity indices, and relationships between microbial biodiversity and soil chemical properties. Using Biolog-ECO methodology to assess microbial metabolic diversity, we aimed to provide a theoretical basis for ecological construction and environmental improvement in the Dushanzi region.

1. Study Area Overview

The study area encompassed native grassland regions surrounding the Dushanzi petrochemical plant. Located at 84°43'–85°06' E and 44°07'–44°23' N at an elevation of approximately 400 m, the Dushanzi District experiences a typical temperate continental climate with an average annual temperature of 8.1°C. Annual precipitation is 108.9 mm, while evaporation reaches 3008.9 mm—nearly 30 times the precipitation. Strong winds in spring and autumn are prominent climatic features. The predominant soil types are desert sierozem and gray-brown desert soil. Dominant spring and summer vegetation includes the constructive species

Artemisia borotalensis (BLH), along with ephemeral plants such as *Eremopyrum triticeum* (HMC) and *Tetracme quadricornis* (SCJ).

2. Experimental Design

The experimental plots were established in areas 2-3 km downwind of the Dushanzi petrochemical plant. Based on vegetation surveys, three dominant herbaceous species from different families were selected according to importance values: *Artemisia borotalensis*, *Eremopyrum triticeum*, and *Tetracme quadricornis*, with importance values of 34.70%, 23.50%, and 11.89%, respectively (hereafter referred to as BLH, HMC, and SCJ). Plots of 3 m × 3 m were randomly established, with distances of 2-3 km between blocks. Each plot contained all three dominant species.

3. Soil Sampling

Soil samples were collected in May 2016. Rhizosphere soil was collected using the shake-off method described by Barber and Riley [13], where soil tightly adhering to roots after gentle shaking was considered rhizosphere soil. Non-rhizosphere soil was collected from areas outside the plant canopy projection, sampled vertically from 0-15 cm depth. In each plot, 3-5 moderately sized plants were randomly selected. Rhizosphere and non-rhizosphere soils were separately mixed, placed in sterile bottles, transported in a portable refrigerator under low temperature, and stored for subsequent functional diversity and physicochemical analyses.

4. Measurement Methods

Soil microbial metabolic activity and functional diversity were measured using the Biolog-ECO microplate method [14]. Fresh soil equivalent to 10 g dry weight was placed in sterile 250 mL flasks, diluted with 90 mL sterile saline solution (0.85%), and shaken for 15 minutes. The supernatant was diluted 1000-fold, and 150 μ L of the diluted bacterial suspension was inoculated into each well of the Biolog-ECO plate using an 8-channel pipette in a laminar flow hood. Plates were incubated at 25°C, and absorbance at 590 nm was read using an ELX-808 microplate reader at regular intervals.

Soil physicochemical properties were measured according to standard methods: soil organic matter (SOM), available phosphorus (AP), electrical conductivity (EC), ammonium nitrogen (NH⁺-N), and nitrate nitrogen (NO⁻-N). Specific procedures followed *Soil Agrochemical Analysis* [15].

5. Data Processing

Microbial metabolic activity was calculated as average well color development (AWCD) using the formula:

$$AWCD = \frac{\sum(C_i - R)}{31}$$

where C_i is the absorbance value at 590 nm for each reaction well, and R is the absorbance of the control well. Wells with $C_i - R < 0$ were recorded as zero in calculations.

Diversity indices were calculated as follows:

Simpson Dominance Index (D):

$$D = \sum P_i^2$$

Shannon Richness Index (H):

$$H = - \sum P_i \times \ln P_i$$

McIntosh Evenness Index (U):

$$U = \sqrt{\sum (C_i - R)^2}$$

where P_i represents the ratio of the difference between the carbon source well and control well absorbance to the total difference across the plate.

Data were analyzed using Microsoft Excel 2010 and SPSS 22.0 software. Analysis of variance (ANOVA), correlation analysis, principal component analysis (PCA), and redundancy analysis (RDA) were performed using Canoco 4.5. All data are presented as means of three replicates.

3. Results

3.1 Variations in Soil Chemical Properties Among Different Plant Rhizosphere and Non-Rhizosphere Soils

Significant differences in soil chemical properties were observed among the three dominant herbaceous plants and between rhizosphere and non-rhizosphere soils (Table 1). For all three species, rhizosphere soil pH was lower than in non-rhizosphere soil, though differences were not significant ($p > 0.05$). Electrical conductivity (EC) was lower in rhizosphere soils, with BLH showing significantly lower EC ($p < 0.05$). Soil organic matter (SOM) and available phosphorus (AP) were significantly higher in rhizosphere soils compared to non-rhizosphere soils

($p < 0.05$). BLH rhizosphere soil exhibited the highest SOM content at 9.70 g/kg and AP content at 31.11 mg/kg. Ammonium nitrogen ($\text{NH}_4\text{-N}$) was significantly lower in rhizosphere soils ($p < 0.05$), while nitrate nitrogen ($\text{NO}_3\text{-N}$) showed no significant differences between rhizosphere and non-rhizosphere soils.

Table 1 Chemical properties of rhizosphere and non-rhizosphere soils of different plant species

Vegetation	Position	pH	EC (S/cm)	SOM (g/kg)	Available P (mg/kg)	$\text{NH}_4\text{-N}$ (mg/kg)	$\text{NO}_3\text{-N}$ (mg/kg)
<i>Artemisia borotalensis</i>	Rhizosphere	8.21 ± 0.01	11.28 ± 0.65	9.70 ± 1.00	21.99 ± 2.75	48.67 ± 4.31	5.29 ± 0.52
	Non-rhizosphere	8.29 ± 0.07	10.74 ± 1.19	9.40 ± 0.57	17.11 ± 4.51	83.29 ± 7.19	4.68 ± 0.06
<i>Eremopyrum triticeum</i>	Rhizosphere	8.24 ± 0.01	11.73 ± 0.63	8.88 ± 0.82	15.27 ± 1.73	52.61 ± 6.63	3.63 ± 0.26
	Non-rhizosphere	8.30 ± 0.07	10.88 ± 1.26	7.26 ± 0.50	17.22 ± 1.05	31.30 ± 2.68	3.29 ± 0.12
<i>Tetradlea cornis</i>	Rhizosphere	8.30 ± 0.01	11.37 ± 0.72	8.28 ± 0.86	16.35 ± 1.68	40.35 ± 6.79	3.56 ± 0.28
	Non-rhizosphere	8.28 ± 0.01	11.34 ± 0.46	9.46 ± 0.37	19.58 ± 1.69	30.98 ± 3.79	3.83 ± 0.07

Note: R = rhizosphere soil; non-R = non-rhizosphere soil. Different lowercase letters indicate significant differences among soil positions for the same plant ($p < 0.05$). Different uppercase letters indicate significant differences between rhizosphere and non-rhizosphere soils for the same plant ($p < 0.05$). Values are means \pm SD.

3.2 Average Well Color Development (AWCD) in Different Plant Rhizosphere and Non-Rhizosphere Soils

AWCD reflects both the quantity and structure of microbial populations in soil. Across all three plant types, AWCD values increased over time, indicating that microbial activity in all soils increased with incubation duration. During the initial 24 hours, AWCD showed minimal change. After 24 hours, AWCD increased sharply, with rhizosphere soils showing faster increases than non-rhizosphere soils. The AWCD ranking at 120 hours was: BLH > SCJ > HMC (Figure 1).

Figure 1 [Figure 1: see original paper] Average well color development (AWCD) of rhizosphere and non-rhizosphere soils of different species over incubation time

3.3 Microbial Diversity Indices in Different Plant Rhizosphere and Non-Rhizosphere Soils

Based on carbon source utilization patterns in rhizosphere and non-rhizosphere soils across different plants, and considering the stabilization trend, data from 120 hours (when optical density stabilized) were selected for microbial community metabolic diversity analysis (Table 2). Simpson dominance index was significantly lower in rhizosphere soils than in non-rhizosphere soils ($p < 0.05$), while Shannon richness index was significantly higher ($p < 0.05$). McIntosh evenness index was significantly lower in rhizosphere soils ($p < 0.05$). BLH rhizosphere soil exhibited the highest microbial community richness and evenness, indicating a broader microbial niche breadth.

Table 2 Functional diversity indices of rhizosphere and non-rhizosphere soil microbial communities under different plant species (120 h)

Plant	Position	Simpson	Shannon	McIntosh
<i>Artemisia borotalensis</i>	Rhizosphere	22.29±1.17 Ba	28.09±4.65 Aa	28.36±3.34 Aa
	Non-rhizosphere	34.98±4.27 Bb	33.93±4.42 Ab	1.88±0.23 Aa
<i>Eremopyrum triticeum</i>	Rhizosphere	28.20±4.03 Bc	1.80±0.15 Aa	1.82±0.34 Aa
	Non-rhizosphere	1.61±0.27 Aa	1.78±0.31 Aa	2.08±0.14 Bb
<i>Tetradme quadricornis</i>	Rhizosphere	1.53±0.28 Aa	1.28±0.09 Aa	1.21±0.86 Aa
	Non-rhizosphere	0.99±0.10 Ab	0.95±0.42 Aa	1.98±0.08 Bc

Note: Different lowercase letters indicate significant differences among soil positions for the same plant ($p < 0.05$). Different uppercase letters indicate significant differences between rhizosphere and non-rhizosphere soils for the same plant ($p < 0.05$).

3.4 Changes in Microbial Metabolic Diversity Patterns in Different Plant Rhizosphere and Non-Rhizosphere Soils

Principal component analysis (PCA) of carbon source utilization by rhizosphere soil microorganisms after 120 hours of incubation revealed three principal components with a cumulative contribution rate of 92.225% (Table 3). PC1 explained 48.515% of variance, with high loadings for carbohydrates, acids, and amines. PC2 explained 30.398% of variance, with significant contributions from

glycyl-L-glutamic acid, D,L- -glycerol phosphate, and phenylethylamine. PC3 explained 13.312% of variance, with major contributions from galacturonic acid, glucosaminic acid, and putrescine. These results indicate that rhizosphere soil microorganisms are particularly sensitive to these carbon source categories.

Table 3 Eigenvalues and cumulative contribution rates of principal components for carbon source utilization by rhizosphere soil microorganisms of three dominant herbaceous plants

Component	Eigenvalue	Contribution Rate (%)	Cumulative Contribution Rate (%)
PC1	15.53	48.52	48.52
PC2	9.73	30.40	78.91
PC3	4.26	13.31	92.23

For non-rhizosphere soils, PCA yielded three principal components with a cumulative contribution rate of 92.470% (Table 4). PC1 explained 63.417% of variance, with major contributions from galacturonic acid, 4-hydroxybenzoic acid, and glucosaminic acid. PC2 explained 17.323% of variance, with high loadings for itaconic acid and putrescine. PC3 explained 11.730% of variance, with significant contributions from glycogen and phenylethylamine. The dominant carbon source utilization pattern in non-rhizosphere soils was primarily acid-based.

Table 4 Eigenvalues and cumulative contribution rates of principal components for carbon source utilization by non-rhizosphere soil microorganisms of three dominant herbaceous plants

Component	Eigenvalue	Contribution Rate (%)	Cumulative Contribution Rate (%)
PC1	20.29	63.42	63.42
PC2	5.54	17.32	80.74
PC3	3.75	11.73	92.47

PCA analysis of carbon source utilization by both rhizosphere and non-rhizosphere soil microorganisms revealed two principal components explaining 87.59% of variance (PC1: 79.67%; PC2: 7.93%). The analysis demonstrated clear spatial differentiation in carbon source utilization between rhizosphere and non-rhizosphere microbial communities, with plant type significantly influencing rhizosphere microbial metabolic characteristics (Figure 2).

Figure 2 [Figure 2: see original paper] Principal component analysis of soil microbial community function at different plants

3.5 Relationships Between Microbial Diversity and Soil Chemical Properties

Correlation analysis between carbon source utilization indices (AWCD, Simpson, Shannon, McIntosh) and soil chemical properties revealed significant relationships (Table 5). AWCD showed significant negative correlations with pH ($p < 0.01$) and significant positive correlations with SOM and AP ($p < 0.05$). Simpson index was significantly negatively correlated with pH ($p < 0.05$) and significantly positively correlated with SOM and AP ($p < 0.05$). Shannon index was significantly negatively correlated with pH ($p < 0.01$) and significantly positively correlated with SOM and AP ($p < 0.05$). McIntosh index showed significant negative correlations with pH and NH₄-N ($p < 0.05$) and significant positive correlations with SOM and AP ($p < 0.05$).

Table 5 Correlations between microbial functional diversity and chemical properties of rhizosphere and non-rhizosphere soils in the petrochemical industrial area

Index	pH	EC	SOM	AP	NH ₄ -N	NO ₃ -N
AWCD	-0.54**	-0.01	0.72**	0.63*	-0.12	-0.03
Simpson	-0.54*	-0.08	0.60*	0.54*	-0.08	-0.06
Shannon	-0.63**	-0.08	0.65**	0.54*	-0.08	-0.05
McIntosh	-0.54*	-0.08	0.60*	0.54*	-0.08	-0.05

Note: * $p < 0.05$; ** $p < 0.01$

4. Discussion

This study employed the Biolog-ECO microplate method to investigate microbial functional diversity in rhizosphere and non-rhizosphere soils of three dominant herbaceous plants in the Dushanzi petrochemical area. The results revealed differences in AWCD, Simpson dominance index, Shannon richness index, and McIntosh evenness index among different plants and soil types. *Artemisia borotalensis* rhizosphere soil exhibited superior microbial metabolic capacity, richness, and diversity compared to other treatments. This may be attributed to *A. borotalensis* having a well-developed taproot and lateral root system, which produces more diverse and abundant root exudates and sloughed root materials, thereby supporting more varied microbial communities while reducing external environmental impacts on microbial functional diversity [17].

Different vegetation types also contributed to observed differences. Studies have shown that vegetation type can influence soil microbial activity and functional composition patterns [18]. Kaiser et al. [19] and Smalla et al. [20] reported that rhizosphere soil microbial diversity varies among different vegetation types,

plant genotypes, and developmental stages within the same genotype. These findings align with our results, suggesting that differences may be related to ecological factors including vegetation composition, root exudates, and soil physicochemical properties [21]. Plant rhizosphere soil microbial functional diversity exhibits temporal and spatial characteristics not only among plant types but also across different growth stages.

Our results also showed that all microbial diversity indices were higher in rhizosphere soils than in corresponding non-rhizosphere soils. This likely occurs because plant roots and residues provide suitable habitats and material resources for rhizosphere microorganisms. Greater carbohydrate secretion by plant roots enhances microbial capacity to utilize carbon substrates [22], consistent with findings from studies on reclaimed red soils [23] and observations of rhizosphere enrichment of soil microbial numbers and enzyme activities [24].

Electrical conductivity was lower in rhizosphere soils than in non-rhizosphere soils, possibly because plant roots absorb or enrich salt-based ions, reducing soil mineral salt ion concentrations. Differential absorption rates of various substances by plant roots create distinct salt content profiles between rhizosphere and non-rhizosphere soils [25, 26]. Our results indicate that *A. borotalensis* and *E. triticeum* can adapt well to saline-alkali conditions, providing favorable environments for soil microorganisms and improving soil fertility.

Correlations between microbial diversity and soil chemical properties revealed that SOM and AP provide essential nutrients and energy for soil microorganisms while preventing dominance of any single microbial population and releasing ecological niches. Appropriate nutrient and salt content in soil can enhance microbial activity and functional diversity. pH emerged as a primary environmental factor inhibiting microbial activity and functional diversity, consistent with previous research [28, 29].

5. Conclusions

Microbial metabolic intensity differed between rhizosphere and non-rhizosphere soils across plant species, with *Artemisia borotalensis* rhizosphere soil showing the highest microbial activity. Rhizosphere soil microorganisms were particularly sensitive to carbohydrates, lipids, acids, and amines, while non-rhizosphere soil microbes showed sensitivity primarily to acids, amino acids, and carbohydrates. Rhizosphere soil microorganisms demonstrated broader carbon source utilization capacity, with distinct profiles characterizing different plant rhizosphere environments. Vegetation presence enhanced soil microbial activity and functional diversity, with better soil conditions favoring environmental adaptation.

Correlation analysis with soil chemical properties identified pH, SOM, AP, and NO₃-N as primary factors influencing soil microbial functional diversity. These

findings indicate that microbial diversity is associated not only with carbon source availability and plant root exudates but also with soil chemical properties. However, deeper relationships and underlying mechanisms require further investigation.

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