Structural Characteristics of Rhizosphere Soil Microbial Communities of Different Desert Plants (Postprint)

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Abstract

In July 2015, rhizosphere soil samples were collected from five typical desert plant species—Ephedra przewalskii, Reaumuria songarica, Sympegma regelii, Nitraria sphaerocarpa, and Salsola passerina—in the Anxi Extremely Arid Desert National Nature Reserve, Gansu Province, and soil microbial community structure was analyzed using the phospholipid fatty acid (PLFA) method combined with the Sherlock microbial identification system. The results demonstrated significant differences in the types and composition of microbial phospholipid fatty acids among the rhizosphere soils of the five desert plants. Specifically, the Gram-positive bacteria-indicating PLFAs 18:0 iso, 16:0 iso, and 17:1 iso 9c were characteristic of Reaumuria songarica and Salsola passerina, while the actinomycete-specific PLFA 18:1 7c 10-methyl occurred exclusively in the rhizosphere of Salsola passerina. Total PLFAs, fungi, actinomycetes, and the fungi/bacteria ratio were significantly highest in Salsola passerina; Gram-positive and Gram-negative bacteria were significantly higher in the rhizospheres of Ephedra przewalskii and Salsola passerina than in other plants; and AM fungi exhibited the highest value in the rhizosphere of Sympegma regelii. Structural equation modeling analysis revealed that soil factors exerted a more pronounced influence on microbial community structure compared with plants, wherein easily extractable glomalin significantly affected actinomycetes, and soil alkali-hydrolyzable nitrogen served as the primary influencing factor for Gram-positive and Gram-negative bacteria. Furthermore, soil microbial community structure could be employed to detect soil degradation status in the rhizosphere microenvironments of different desert plants.

Full Text

Characteristics of Microbial Community Structure in Rhizosphere Soil of Different Desert Plants

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Abstract: To effectively measure and manage plant growth in arid desert regions, an improved understanding of spatial patterns of desert soil resources and the role of soil microbes is required. Rhizosphere soils of *Sympegma regelii*, *Nitraria sphaerocarpa*, *Salsola passerina*, *Ephedra przewalskii*, and *Reaumuria songarica* were collected in July 2015 from the Anxi Extreme-Arid Desert National Nature Reserve in Gansu Province, China. Soil microbial community structure in the rhizosphere of these five desert plants was analyzed using phospholipid fatty acid (PLFA) analysis combined with the Sherlock microbial identification system. The results showed that soil microbial PLFA species and composition in the rhizosphere of the five desert plants differed significantly. The characteristic PLFAs of Gram-positive bacteria—18:0 iso, 16:0 iso, and 17:1 iso 9c—were unique to the rhizosphere of *S. passerina*. The PLFA 18:1 7c 10-methyl, which indicates actinomycetes, existed only in the rhizosphere of *S. passerina*. Total PLFAs, fungi, actinomycetes, and the fungi/bacteria ratio were highest under *S. passerina*. However, Gram-positive and Gram-negative bacteria were significantly higher under *E. przewalskii* and *S. passerina* than under other plants. AM fungi had the highest value under *S. regelii*. Structural equation model analysis indicated that, compared with plants, soil factors had more significant effects on microbial community structure. Easily extracted glomalin had a significant effect on actinomycetes. Soil available nitrogen was the major influencing factor for Gram-positive and Gram-negative bacteria. Moreover, soil microbial community structure can be used to monitor soil degradation in the rhizosphere microenvironment of different desert plants.

Keywords: soil microbial community; phospholipid fatty acid (PLFA); desert plant; Anxi Extreme-arid Desert

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1. Sample Site Overview

The study area is located in the Anxi Extreme-Arid Desert National Nature Reserve in Gansu Province (94°45 -97°00 E, 39°52 -41°53 N), which has a typical continental climate. Annual rainfall is less than 52.0 mm, unevenly distributed across seasons, with summer rainfall accounting for most of the precipitation. The region has 7.8-10℃ of temperature variation. Soil types are primarily gray-brown desert soils with low vegetation coverage, dominated by *Reaumuria songarica*, *Ephedra przewalskii*, *Sympegma regelii*, and *Salsola passerina*. These five species, belonging to Ephedraceae, Tamaricaceae, Amaranthaceae, and Zygophyllaceae families, represent typical vegetation of the Anxi extreme-arid desert and exhibit significant differences in regulating soil microenvironments. All five plants have well-developed root systems and wind-sand resistance characteristics that are important for community development and maintenance of the extreme-arid desert ecosystem.

The basic information of five plant communities

2. Sample Collection

In July 2015, five typical plant communities were selected in the reserve. Using a five-point sampling method, five plants with similar growth status were chosen from each community. After removing the surface litter layer, root samples were collected within 0–30 cm soil depth at 0–30 cm distance from the main stem. Soils attached to roots were shaken off as rhizosphere soil, mixed uniformly, and placed in insulated ziplock bags for transport to the laboratory. A portion was stored in a refrigerator for microbial analysis, while the remainder was used for soil physicochemical property determination.

3. Soil Factor Determination

Soil pH was measured with a precision pH meter (PHS-3C). Available phosphorus was extracted with sodium bicarbonate and determined by molybdenum-antimony anti-colorimetry using a Smartchem200 auto-analyzer. Alkali-hydrolyzable nitrogen was measured by the alkali diffusion method. Soil organic carbon was determined by muffle furnace drying. Total and easily extracted glomalin were measured using the method of Wright and Upadhyaya [9] and Janos et al. [10]. Acid and alkaline phosphatase activities were determined using the method of Tabatabai [11] by measuring the conversion of p-nitrophenyl phosphate disodium.

4. Soil Microbial Phospholipid Fatty Acid Determination

Soil microbial community structure was determined according to the method of Bossio et al. [12]. Lipids were extracted from freeze-dried soil using a chloroform:methanol:phosphate buffer solution (1:2:0.8 v/v/v). Lipid classes were separated and purified using silica columns (SPE-Si, 500 mg/6 mL). Purified fatty acids were methylated with methanol solution and C19:0 internal standard, then detected and analyzed using an Agilent 6890N GC-MS analyzer combined with the Sherlock Microbial Identification System (MIS4.5). The PLFA classification is shown in Table 2.

Classifications of phospholipid fatty acid (PLFA) signatures

5. Data Analysis

All data were averaged from three replicates. Excel 2010 was used for data organization. One-way ANOVA was performed on soil microbial communities using SPSS 19.0. Principal component analysis (PCA) was conducted on soil factors and microbial communities. Correlations between soil factors and microbial communities were determined. Amos Graphics 22.0 was used to construct structural equation models (SEM) with the following fitting criteria: ^{2}/df $>$ 0.05, RMSEA $<$ 0.05.

1. Soil Physicochemical Properties

Soil organic carbon was highest in *S. regelii* rhizosphere, while available phosphorus showed no significant differences among plants. Alkaline phosphatase had maximum values in *R. songarica* and *N. sphaerocarpa*, with the lowest value in *E. przewalskii*. Acid phosphatase was significantly higher in *R. songarica* than in other plants, with no significant differences among the others. Ammonia nitrogen differed significantly among plants, with the lowest value in *N. sphaerocarpa*. Total glomalin was significantly higher in *N. sphaerocarpa* than in *E. przewalskii*, while easily extracted glomalin showed no significant differences among plants.

Soil factors in the rhizosphere of different plants

2. Soil Microbial Phospholipid Fatty Acid Analysis

This study selected PLFAs greater than 0.01 nmol/g for analysis. Principal component analysis showed that the first three principal components explained 96.838% of the variance (77.140%, 12.617%, and 7.081% respectively), indicating significant differences in soil microbial composition among different plants. *N. sphaerocarpa* had the highest scores on PC1 and PC2, while *R. songarica* and *S. passerina* were more dispersed in distribution. The five plant species showed no significant differences in scores on the three principal components.

The main PLFAs in *E. przewalskii* rhizosphere soil were those indicating Gram-negative bacteria: 16:1 7c, 17:1 8c, 17:0 cyclo 7c, 16:0 2OH, 18:1 7c, 19:1 6c, and 19:0 cyclo 7c. In *R. songarica*, the dominant PLFAs were 18:0 iso and 20:4 6c (indicating eukaryotes), and 23:1 4c (only present in *R. songarica*). *S. regelii* rhizosphere soil was dominated by PLFAs indicating Gram-negative bacteria (16:1 7c, 18:1 7c, 19:0 cyclo 7c), actinomycetes (16:0 10-methyl), and eukaryotes (16:3 6c, 18:3 6c). *S. passerina* had characteristic PLFAs including 15:0 iso, 15:0 anteiso, 17:0 iso, 16:0 iso, 17:1 iso 9c (Gram-positive bacteria); 16:1 7c, 17:1 8c, 18:1 7c, 19:0 cyclo 7c (Gram-negative bacteria); 18:1 7c 10-methyl (unique to *S. passerina*); 16:0 10-methyl, 18:0 10-methyl (actinomycetes); and 20:4 6c (eukaryotes). The main PLFAs in *N. sphaerocarpa* were dominated by those indicating Gram-negative bacteria.

Scores of different plants in PC1, PC2 and PC3

3. Soil Microbial Community Structure Analysis

The microbial community structure differed significantly among the five plant rhizosphere soils. Gram-negative bacteria were significantly higher than other microorganisms in *R. songarica*, followed by Gram-positive bacteria, actinomycetes, and eukaryotes. In *N. sphaerocarpa* and *S. passerina*, Gram-negative bacteria were significantly higher than other groups. In *S. regelii*, fungi were significantly highest, while Gram-negative and Gram-positive bacteria followed. Bacteria were significantly higher in *S. passerina* than in other plants, with no significant differences among the other plants. Total PLFAs, fungi, actinomycetes, and fungi/bacteria ratio were highest in *S. passerina.* Gram-negative and Gram-positive bacteria were highest in *E. przewalskii* and *S. passerina*, significantly higher than in other plants.

Soil microbial PLFAs concentration of different plants

4. Correlation Analysis Between Soil Microbial Community and Soil Factors

Correlation analysis showed that pH was significantly negatively correlated with Gram-negative bacteria and significantly positively correlated with fungi. Available phosphorus was significantly positively correlated with Gram-positive bacteria, actinomycetes, and fungi. Ammonia nitrogen was significantly positively correlated with Gram-positive bacteria and actinomycetes. Acid phosphatase was significantly negatively correlated with Gram-positive bacteria and actinomycetes. Easily extracted glomalin was significantly positively correlated with Gram-negative bacteria, Gram-positive bacteria, and actinomycetes. Alkali-hydrolyzable nitrogen was significantly negatively correlated with Gram-negative bacteria, Gram-positive bacteria, and actinomycetes. Total glomalin was significantly negatively correlated with fungi.

The correlation between soil microbial communities and soil factors

5. Effects of Plant Species and Soil Factors on Microbial Communities

Based on correlation coefficients, a structural equation model was constructed to quantify relationships among plants, easily extracted glomalin, alkali-hydrolyzable nitrogen, Gram-negative bacteria, Gram-positive bacteria, and fungi. The model showed that easily extracted glomalin had extremely significant direct effects on actinomycetes. Alkali-hydrolyzable nitrogen was the main influencing factor for Gram-positive and Gram-negative bacteria. Plants had indirect but non-significant effects on Gram-positive bacteria and fungi through soil factors. The model fit the following criteria: $^2 = 8.018$, df = 16, P = 0.948, RMSEA = 0, GFI = 0.883.

[Figure 2: see original paper] Structural equation model about soil microbial communities, plants and soil factors

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1. Discussion

The rhizosphere soils of the five desert plants were dominated by characteristic phospholipid fatty acids indicating Gram-negative bacteria, Gram-positive bacteria, and actinomycetes. The PLFAs 18:0 iso, 16:0 iso, and 17:1 iso 9c, which characterize Gram-positive bacteria, were unique to *R. songarica* and *S. passerina*. The 18:1 7c 10-methyl PLFA, which indicates actinomycetes, existed only in *S. passerina*. These unique characteristic PLFAs demonstrate that different plant species have significantly different soil microbial species and composition, which are sensitive to soil environmental changes. Therefore, plant-mediated changes in soil properties directly affect soil microbes.

Reaumuria songarica is a typical salt-secreting plant that can reduce soil salinity through salt transfer, while *Salsola passerina* is a salt-accumulating plant. Zuo Yiling et al. [15] showed that Gram-positive bacteria dominate in desert arid environments. However, this study found Gram-negative bacteria dominated the rhizosphere soils of the five plants, which differs from previous research [13]. This discrepancy may be related to sampling time. July is the vigorous growth period for plants when photosynthesis and physiological metabolism are enhanced, root secretions increase, and soil organic matter content rises, favoring Gram-negative bacteria growth. Gram-negative bacteria can rapidly grow in nutrient-rich environments [16].

The microbial community structure differed significantly among the five desert plant rhizosphere soils, likely related to root exudates. Different root exudates have different physicochemical properties that stimulate soil microbes differently [5]. Gram-negative and Gram-positive bacteria showed similar trends, being highest in *S. passerina*, followed by *E. przewalskii*, *N. sphaerocarpa*, *S. regelii*, and lowest in *R. songarica*. This indicates that *S. passerina* rhizosphere soil environment is more conducive to microbial growth. *Salsola passerina* had higher soil organic matter content, which can further improve its rhizosphere soil environment. Aguilera et al. [18] demonstrated that soil microbial abundance increases with available nutrients. Total PLFAs, bacteria, actinomycetes, and fungi were highest in *S. passerina*, indicating its soil microbial content and rhizosphere soil quality are relatively highest.

Fungi were highest in *S. regelii* and lowest in *E. przewalskii*. As plant symbiotic fungi, fungal occurrence and distribution often depend on plant roots [19] and are sensitive to soil salinity [20]. *Ephedra przewalskii* is an extremely drought-tolerant evergreen shrub with tall plants and taproot systems, while *S. regelii* is a small shrub. Therefore, nutrient input to soil from *E. przewalskii* may be far lower than from *S. regelii*, resulting in significantly lower rhizosphere soil microbial content. The salt-accumulating characteristics of *E. przewalskii* may inhibit certain fungal infections and symbiosis.

Compared with plants, soil factors had more significant effects on soil microbial community structure, with easily extracted glomalin and alkali-hydrolyzable nitrogen being the main influencing factors. This may be related to the ecological environment of the sample plots. The Anxi extreme-arid desert has low rainfall, and July temperatures reach the annual maximum. Plant litter and root exudate decomposition are suppressed, leaching is reduced, and nutrients from plant decomposition have no significant effect on soil microbes [22]. Glomalin is a glycoprotein secreted by AM fungi that is released into soil through hyphal and spore degradation. It is an important component of soil organic carbon pools, and its adhesive properties benefit soil aggregate formation and improve soil aeration [23], providing a suitable growth environment for actinomycetes. Alkali-hydrolyzable nitrogen had significant negative effects on Gram-negative and Gram-positive bacteria, which may be related to how different carbon-nitrogen ratios affect bacteria. As an important component of bacterial cells, higher nitrogen content leads to stronger bacterial synthesis. However, desert soils have low organic carbon content that cannot meet bacterial energy demands for synthesis, and excessive nitrogen leads to ammonium salt accumulation that inhibits bacterial growth and organic matter decomposition.

2. Conclusion

The rhizosphere soil microbial community structure of the five desert plants differed significantly and was closely related to plant communities and soil factors. However, soil factors had more significant effects than plants, with easily extracted glomalin and alkali-hydrolyzable nitrogen being the main influencing factors. This not only helps reveal the relationship between desert plants, soil factors, and soil microbial communities, but also provides important significance for desert vegetation restoration using biotechnology.

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