

## Effects of Early-Stage Vegetation Reconstruction on Soil Microbial Community Structure in Arid Sandy Areas (Postprint)

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### Abstract

To reveal the effects of vegetation reconstruction on soil microbial community structure in arid aeolian sandy regions, soils from the vegetation reconstruction area of the ecological protection system along the Tengger Desert section of the Wuhai-Maqin Expressway were studied, with mobile dunes serving as a control. High-throughput sequencing technology was utilized to investigate the variation characteristics of soil microbial community structure in the vegetation reconstruction area following vegetation restoration and reconstruction. The results indicated that the soil microbial community composition was similar between the vegetation reconstruction area and the shifting sand area, with Actinobacteria (58.53%~67.85%) and Proteobacteria (16.53%~19.68%) being the dominant phyla in both. The dominant genera included *Nocardia*, *Methylocella*, *Rubrobacter*, and *Microvirga*. Compared with the shifting sand control, the vegetation reconstruction area significantly increased the relative abundance and diversity of most soil bacterial phyla. Following vegetation restoration and reconstruction, soil pH, electrical conductivity, and salt content decreased; soil microbial biomass phosphorus (MBP), soil microbial biomass carbon (MBC), and the soil microbial biomass carbon to nitrogen ratio (MBC:MBN) in shallow soil layers (0~5 cm, 5~10 cm, and 10~20 cm) were significantly higher in the vegetation reconstruction area than in the shifting sand area. Therefore, for desert highway ecological protection systems, vegetation reconstruction can significantly improve shallow soil nutrient status, microbial relative abundance, and diversity levels, and constitutes an important measure for improving the desert ecological environment.

## Full Text

### Effects of Short-Term Vegetation Reconstruction on Soil Microbial Community Structure in Arid Desert Areas

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#### Abstract

To elucidate the effects of vegetation reconstruction on soil microbial community structure in arid desert regions, this study examined soils from the revegetation area of the ecological protection system along the Wuhai-Maqin Highway crossing the Tengger Desert, using moving dunes as a control. High-throughput sequencing technology was employed to investigate the changing characteristics of soil microbial community structure during vegetation restoration and reconstruction. Results showed that microbial community composition was similar between the revegetated soils and moving dunes, with both dominated by Actinobacteria (58.53%-67.85%) and Proteobacteria (16.53%-19.68%). Dominant genera included *Nocardioides*, *Methylothera*, *Rubellimicrobium*, and *Microvirga*. Compared with the moving dune control, vegetation reconstruction significantly increased the relative abundance and diversity of most bacterial phyla. Following vegetation restoration, soil pH, electrical conductivity, and salinity content decreased, while soil microbial biomass phosphorus (MBP) and the microbial biomass carbon-to-nitrogen ratio (MBC:MBN) were significantly higher in the revegetated area. These findings demonstrate that for desert highway ecological protection systems, vegetation reconstruction can significantly improve shallow soil nutrient status, microbial relative abundance, and diversity levels, representing an important measure for improving desert ecological environments.

**Keywords:** arid windy sand area; vegetation reconstruction; microbial community structure

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#### Introduction

Arid regions constitute approximately one-third of China's total land area and are characterized by extremely harsh natural environments. Combined with

long-term overexploitation of resources and global climate change, these factors have intensified land desertification and caused severe ecological degradation in these areas [1,7-8]. Preventing further degradation has become an urgent priority. Vegetation reconstruction has proven to be an effective approach for ecological restoration of desertified land in China's arid regions, capable of improving soil fertility, reducing wind-sand hazards, and promoting overall ecological improvement. During vegetation restoration, changes in vegetation coverage affect soil microbial abundance and activity, which in turn alters soil physicochemical properties, while improved nutrient conditions subsequently promote vegetation growth [4-5,11-12]. Soil microorganisms, vegetation, and physicochemical properties interact synergistically and influence one another [20,22,25].

Soil microorganisms constitute an essential component of soil systems, participating in mineralization of organic matter, formation of humus, and nutrient cycling processes, while playing crucial roles in stabilizing soil structure and improving physicochemical properties [13,27]. Soil microbial communities respond more sensitively to environmental changes than soil physicochemical properties, and alterations in their community structure and function can effectively indicate early changes in ecosystem function [1,7-8]. Previous research has demonstrated that vegetation restoration and reconstruction exert certain influences on soil microorganisms [4-5,11-12]. As vegetation gradually recovers, soil structure and nutrient status improve to varying degrees, microbial richness and diversity increase significantly, and microbial biomass increases with restoration age while decreasing with soil depth [4-5,11-12]. Lü Xingyu et al. [13] studied the three major microbial groups (fungi, bacteria, and actinomycetes) in the Shapotou sand-fixing vegetation area and found that microbial quantity recovery curves showed an initial increase followed by a decrease, peaking at 18-24 years and reaching 31.6%-83.7% of natural vegetation levels after 24-36 years.

Currently, soil microorganisms have become one of the key indicators for evaluating vegetation restoration and reconstruction processes [20,22,25]. Domestic scholars have conducted extensive theoretical and practical research on ecological restoration in deserts, grasslands, wetlands, and forests of arid and semi-arid regions, including studies on the effects of vegetation reconstruction and restoration on soil habitats and microbial community structure and function. However, due to differences in climate, soil, and vegetation reconstruction measures, targeted systematic research is required for specific study objects. Therefore, this study selected the vegetation reconstruction area of the ecological protection system along the Wuhai-Maqin Highway in the southeastern margin of the Tengger Desert as the research region, using adjacent moving dunes as a control. High-throughput sequencing was employed to investigate the composition and changes of soil microbial community structure during the initial stage of vegetation reconstruction, aiming to explore the effects of vegetation reconstruction on soil microbial community structure in arid desert areas and provide scientific support for vegetation reconstruction and ecological restoration in such regions.

## 1. Study Area Overview

The study area is located in the southeastern margin of the Tengger Desert, in the artificial vegetation reconstruction zone along the Zhongwei section of the Wuhai-Maqin Highway (referred to as the Wuma Highway) (37°32' N, 105°02' E). As the first highway crossing the hinterland of the Tengger Desert, the Wuma Highway traverses approximately 21 km of the desert, with strong wind-sand activities on both sides. Wind speeds exceeding the sand-moving threshold of  $5 \text{ m} \cdot \text{s}^{-1}$  occur on about 200 days annually, and the dunes are predominantly moving dunes with an average elevation of 1339 m. The region has an annual average temperature of 9.6 °C, annual precipitation of approximately 186 mm, a minimum temperature of -24.5 °C, and a maximum temperature of 38.1 °C [FIGURE:N]. To ensure safe operation of the Wuma Highway, an ecological protection system approximately 300 m wide was established on both sides of the road. The main artificial sand-fixing shrubs in this area include *Caragana korshinskii*, *Hedysarum scoparium*, and *Hedysarum mongolicum*.

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## 2. Research Methods

The revegetation area (test road section) of the Wuma Highway ecological protection system was established in early 2019, with *Caragana korshinskii* as the constructive species planted at low density (2 m × 3 m spacing). Sampling was conducted in late July 2020. Three 10 m × 10 m plots were established in both the artificial vegetation reconstruction area and adjacent moving dune area. Five sampling points were randomly selected in each plot, and soil samples were collected from five layers: 0–5 cm, 5–10 cm, 10–20 cm, 20–50 cm, and 50–100 cm. Soil samples from the same layer within each plot were mixed to form one composite sample. Each soil sample was divided into two portions: one was air-dried indoors, cleared of residual litter, and passed through a 2 mm sieve for determination of soil physicochemical properties; the other was stored at -80 °C for microbial biomass detection and high-throughput sequencing.

Soil pH was measured using a potentiometric method; electrical conductivity (EC) was determined with a conductivity meter; soil microbial biomass carbon (MBC) was extracted using chloroform fumigation- $\text{K}_2\text{SO}_4$  extraction and measured with a fully automatic organic carbon-nitrogen analyzer (Elementar vario TOC/TNb Analyzer, Germany); soil microbial biomass nitrogen (MBN) was measured using the same method; and soil microbial biomass phosphorus (MBP) was extracted with  $\text{NaHCO}_3$  [13].

For microbial community analysis, soil microbial DNA was extracted using the Omega Bio Z.N.A. Soil DNA Kit. PCR amplification was performed using the TransGen AP221-02 kit with the following 20 L reaction system: 2.0 L of 10×buffer, 0.8 L of dNTPs ( $5 \text{ mmol} \cdot \text{L}^{-1}$ ), 0.8 L of forward primer (5'-GTGCCAGCMGCCGCGG-3',  $5 \times 10 \text{ mol} \cdot \text{L}^{-1}$ ), 0.8 μL of reverse primer (5'-CCGTCAATTCMTTTRAGTTT-3',  $5 \times 10 \text{ mol} \cdot \text{L}^{-1}$ ).

1}), 0.4  $\mu$ L of *FastP fupolymerase*, 10 ng of template DNA, and dH<sub>2</sub>O added to 20  $\mu$ L. Reaction conditions were: 10 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 55 °C, and 45 s at 72 °C, with a final extension of 10 min at 72 °C. PCR products were purified using the AxyPrep DNA Gel Extraction Kit and quantified with the QuantiFluor™ fluorescence quantification system. Equal proportions of each sample were mixed and subjected to paired-end sequencing on the Illumina MiSeq platform following standard protocols.

Raw sequences were processed by merging paired-end reads based on overlap and performing quality control to obtain clean reads. Effective sequences were clustered into operational taxonomic units (OTUs) at 97% similarity, followed by chimera filtering. Representative sequences from each OTU were selected and compared against reference databases for species annotation. The relative abundance of each species was calculated, and alpha diversity indices were computed for each sample.

Soil physicochemical properties and bacterial community composition were analyzed using JMP 10.0 and SPSS 26.0 software. Redundancy analysis (RDA) was performed using CANOCO 5.0, and curves were fitted and plotted using Origin 19.0 (Origin Lab Corp.).

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### 3. Results

**3.1 Changes in Soil Microbial Community Structure with Vegetation Reconstruction** At the phylum level, soil microorganisms in the study area were dominated by Actinobacteria, Chloroflexi, Proteobacteria, Acidobacteria, Firmicutes, Bacteroidetes, Cyanobacteria, Gemmatimonadetes, Deinococcus-Thermus, and Spirochaetes [Figure 1: see original paper]. Both the revegetation area and moving dune area shared the same dominant bacterial phyla: Actinobacteria (58.53%–67.85%) and Proteobacteria (16.53%–19.68%), which together accounted for more than 75% of total sequences. Compared with the moving dune area, the revegetation area showed higher relative abundances of Proteobacteria, Chloroflexi, Acidobacteria, Firmicutes, Bacteroidetes, Cyanobacteria, Gemmatimonadetes, and Spirochaetes, but lower relative abundances of Actinobacteria and Deinococcus-Thermus.

At the genus level, the dominant genera (relative abundance >0.5%) included *Deinococcus*, *Enhydrobacter*, *Flavisolibacter*, *Rubellimicrobium*, *Methylothermus*, *Agromyces*, *Microvirga*, *Rhizobium*, *Marmoricola*, and *Nocardioideus*. Compared with the moving dune area, the revegetation area had lower relative abundances of *Deinococcus*, *Enhydrobacter*, and *Flavisolibacter* (with *Enhydrobacter* being only 0.26 times that of the moving dune), but higher relative abundances of *Rubellimicrobium*, *Methylothermus*, *Agromyces*, *Microvirga*, *Rhizobium*, *Marmoricola*, and *Nocardioideus*, with *Rhizobium* reaching 3.29 times that of the moving dune.

**3.2 Characteristics of Microbial Community Structure Changes with Soil Depth** At the phylum level, in the moving dune area, the relative abundance of Actinobacteria showed an initial increase followed by a decrease with soil depth, while Proteobacteria showed the opposite pattern. In the revegetation area, no significant pattern was observed for these two phyla. Compared with the moving dune area, the revegetation area showed fluctuating changes with soil depth: Actinobacteria relative abundance increased by 10.70% at 5-10 cm but decreased by 37.28% at 10-20 cm compared to the moving dune; Proteobacteria relative abundance showed opposite trends, increasing by 30.74% at 0-5 cm, 51.21% at 5-10 cm, and 4.55% at 10-20 cm, but decreasing by 9.14% at 20-50 cm and 56.13% at 50-100 cm. Additionally, Cyanobacteria relative abundance in the revegetation area was 2.22 times higher at 0-5 cm than in the moving dune, but no more than 100% higher in other layers. Overall, most dominant soil microorganisms in each layer of the revegetation area had higher relative abundances than those in the moving dune.

At the genus level, compared with the moving dune area, the revegetation area showed significant differences. *Marmoricola*, *Agromyces*, *Rubellimicrobium*, and *Flavisolibacter* in the 0-5 cm layer were 1.26-2.44 times those in the moving dune; *Deinococcus* and *Microvirga* in the 10-20 cm layer were 0.81-1.24 times those in the moving dune; and *Enhydrobacter* and *Nocardioides* in the 20-50 cm layer were 0.44-1.00 times those in the moving dune.

**3.3 Differences in Soil Microbial Species Numbers Between Revegetation and Moving Dune Areas** At the species level, differences existed among soil layers within the same region. In the moving dune area, species numbers across layers (0-5 cm, 5-10 cm, 10-20 cm, 20-50 cm, 50-100 cm) showed a decreasing then increasing trend. The revegetation area showed the same pattern, with species numbers in each layer being higher than those in corresponding layers of the moving dune. The revegetation area had 329.17 times more species in the 0-5 cm layer than the moving dune. The Chao1 and Shannon indices were higher in the revegetation area than in the moving dune for all layers except 50-100 cm, where only the Shannon index was higher.

**3.4 Differences in Soil Microbial Diversity Between Revegetation and Moving Dune Areas** Microbial diversity indices, including the Chao1 richness index, Shannon diversity index, and Simpson diversity index, reflect species abundance in biological communities. The results showed no overlap between soil samples from different layers, with the revegetation and moving dune areas forming two distinct groups [Figure 4: see original paper]. Samples from the moving dune area were distributed relatively close to each other, while samples from the revegetation area were more dispersed, particularly in the 20-50 cm and 50-100 cm layers [Figure 5: see original paper].

Following vegetation reconstruction, significant changes occurred in soil physicochemical properties and microbial biomass. Compared with the moving dune

area, vegetation restoration decreased overall pH, electrical conductivity (EC), and salinity content. Soil MBC:MBN was highest in the surface layer (0-5 cm), being 2.18 times that of the moving dune. MBP in the revegetation area was  $25.26 \text{ mg} \cdot \text{kg}^{-1}$ ,  $392.87 \text{ mg} \cdot \text{kg}^{-1}$ , and  $144.19 \text{ mg} \cdot \text{kg}^{-1}$  in the 0-5 cm, 5-10 cm, and 10-20 cm layers, respectively, representing increases of 2.31, 1.48, and 1.25 times compared with the moving dune. MBC and MBN in the revegetation area were higher than those in the moving dune across all layers. Overall, MBC:MBN in the surface and middle layers (0-5 cm, 5-10 cm, and 10-20 cm) was higher in the revegetation area than in the moving dune .

To reveal relationships between soil physicochemical properties, microbial biomass, and microbial diversity, redundancy analysis (RDA) was performed with microbial diversity as the response variable and soil properties and microbial biomass as explanatory variables [Figure 7: see original paper]. The results showed that microbial diversity was closely related to soil physicochemical properties and microbial biomass, with different factors having varying explanatory power. In the moving dune area, the first and second axes explained 87.40% and 11.72% of the variation, respectively, with MBC:MBN, pH, MBN, Eh, MBP, salinity, EC, and MBC having significant effects on microbial diversity (pH > MBN > Eh > MBP > salinity > MBC:MBN > EC > MBC). In the revegetation area, the first and second axes explained 90.56% and 7.49% of the variation, respectively, with EC, pH, Eh, MBC, salinity, MBN, MBC:MBN, and MBP significantly affecting microbial diversity (EC > pH > Eh > MBC > salinity > MBN > MBC:MBN > MBP). Notably, MBC:MBN was positively correlated with microbial diversity in the revegetation area but negatively correlated in the moving dune area.

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#### 4. Discussion

This study found that soil microbial community composition was similar between the moving dune and revegetation areas, with both dominated by Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria, Firmicutes, Bacteroidetes, Cyanobacteria, Deinococcus-Thermus, Gemmatimonadetes, and Spirochaetes. However, relative abundances differed, with most soil microbial phyla showing higher relative abundance in the revegetation area than in the moving dune. Numerous studies have identified Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes as the main bacterial groups in desert soils [13,27]. Actinobacteria typically dominate desert regions [20,22,25] as thermophilic and weakly alkaliphilic bacteria that can degrade cellulose and lignin in plant residues, providing nutrients to soil [13,27]. Their filamentous growth through spores enables them to tolerate UV radiation and damage from drought and high temperatures, allowing adaptation to harsh wind-sand environments. Proteobacteria are dominant in most soils, and their abundance is positively correlated with soil nitrogen cycling and plant photosynthesis intensity [4-5,11-12]. Following vegetation reconstruction,

plant litter is continuously decomposed by microorganisms and enters the soil, increasing nutrient content. Proteobacteria utilize organic nitrogen to enhance soil nitrogen fixation, and increased soil organic carbon and total nitrogen provide nutritional conditions for their growth and reproduction, leading to continuously increasing abundance [13,27].

The desert environment is characterized by aridity and low rainfall, with soil microorganisms primarily using plant residues as a nutrient source. During plant growth, root exudates provide various ions and compounds that offer abundant nutrients for microorganisms. Soil microorganisms further decompose litter, increasing soil organic matter and other nutrients, which in turn promotes microbial reproduction [1,7-8]. In contrast, the moving dune area has virtually no vegetation cover and lacks nutrient sources such as root exudates and above-ground litter compared with the revegetation area, thereby limiting microbial growth and reproduction [4-5,11-12]. Additionally, vegetation reconstruction affects soil microorganisms by influencing soil moisture, nutrients, and temperature. Studies have shown that soil microbial numbers are positively correlated with plant litter, vegetation coverage, and organic matter content [20,22,25].

At the soil microbial genus level, the revegetation area had higher relative abundances of *Rubellimicrobium*, *Methylothera*, *Agromyces*, *Microvirga*, *Rhizobium*, *Marmoricola*, and *Nocardioides* than the moving dune area, with *Rhizobium* reaching 3.29 times that of the moving dune. The *Caragana korshinskii* planted in the study area is a legume that can form a symbiotic nitrogen-fixing system with rhizobia, thereby improving the nutrient-poor conditions of the aeolian sandy soil.

Soil microbial diversity and abundance reflect ecosystem health and stability [13,27]. Research indicates that microbial diversity is influenced by both biotic and abiotic factors, with plants not only providing nutrient-rich habitats but also affecting selective pressures on soil microorganisms [1,7-8]. Studies in the Kubuqi Desert have shown that vegetation reconstruction significantly increased soil bacterial diversity and abundance compared with moving dunes because the low nutrient content in moving dune soils limits microbial development, while establishment of sand-fixing vegetation increases soil fine particles, plant litter return, and root exudates, enhancing soil nutrients and promoting gradual increases in microbial biomass [4-5,11-12]. Yang Ruihong et al. [20,22,25] found that desert vegetation not only harbors rich soil bacterial diversity but also contains a certain number of new microbial species, with vegetation type and climate change all affecting soil microbial diversity.

Soil microbial biomass can reflect ecosystem soil fertility and plant productivity to some extent and responds very sensitively to environmental changes [13,27]. Different vegetation restoration methods and growth stages have varying effects on soil microbial carbon, nitrogen, and phosphorus, with different vegetation communities having significant impacts on microbial biomass carbon, nitrogen, and phosphorus [4-5,11-12]. Soil organic matter and total nitrogen are the main sources of microbial biomass carbon and nitrogen, while temperature and mois-

ture are important limiting factors for soil microorganisms. Changes in temperature and moisture affect the decomposition and transformation of organic matter, thereby influencing soil microbial utilization of organic matter and nitrogen [1,7-8]. During normal plant growth and development, metabolites and litter are continuously released into the environment, while root exudates continuously exchange ions with the soil solution, promoting material cycling and energy flow between roots and the soil ecosystem [20,22,25].

This study found that compared with the moving dune area, soil MBC:MBN in the revegetated area was higher in the surface and middle layers (0-5 cm, 5-10 cm, and 10-20 cm). Soil microorganisms exhibit vertical distribution patterns. The initial vegetation restoration period was relatively short, with more developed root systems in the surface soil and litter input providing relatively abundant nutrients and growth space for soil microorganisms, allowing them to proliferate. In deeper layers, fewer plant roots exist, no litter input occurs, and increased soil bulk density reduces permeability, creating unfavorable conditions for microbial survival and reproduction [13,27]. As restoration age increases, plant roots develop deeper into the soil, gradually improving the physicochemical status and nutrient environment of deep soil layers, which promotes microbial community distribution to deeper layers. Long-term ecological restoration results show that vegetation at different restoration stages differentially affects soil environmental microorganisms. For example, in the early restoration stage, *Artemisia ordosica* is more conducive to recovery of microorganisms involved in nitrogen cycling (ammonifying and nitrifying bacteria), while in later stages, *Caragana korshinskii* is more beneficial for improving cellulose-decomposing bacteria [13,27].

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## 5. Conclusions

The soil microbial community composition was similar between the revegetation and moving dune areas, with both dominated by Actinobacteria and Proteobacteria as the dominant groups. Dominant genera included *Nocardioidea*, *Methylobacterium*, *Rubellimicrobium*, and *Microvirga*, with taxonomically undefined rare bacterial groups accounting for a high proportion of total sequences. Most phyla showed significantly higher abundance and diversity in the revegetation area than in the moving dune area. Vegetation restoration and reconstruction significantly improved soil chemical properties and increased soil microbial biomass carbon, nitrogen, and phosphorus. The restoration and improvement of soil habitats and microbial communities are key indicators of ecological restoration in arid desert areas and provide an important basis for evaluating the stability of artificial vegetation structure and function.

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