Effects of Mine Water on Soil Microbial Community Structure and Diversity in Desert Steppe: Postprint

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Abstract

To investigate the effects of mine water discharge on soil microbial community structure and diversity in desert steppe, this study took the desert steppe at a mine water discharge site in the Ningxia arid wind-sand region as the research object. Through high-throughput sequencing analysis of bacterial and fungal community composition in soil layers of 0–10 cm, 10–20 cm, and 20–30 cm from the lakeside waterfront area (MJTA), near-shore terrestrial area (MJTB), and natural area (MJTC) of the mine water discharge lake, combined with habitat factors such as soil physicochemical parameters, the main factors influencing the effects of mine water disturbance on soil microbial community structure composition and diversity were revealed. The results showed: (1) Mine water discharge significantly altered the community composition of soil bacteria and fungi, and exerted significant effects on the differences in relative abundance at the phylum level of soil bacterial and fungal communities. (2) The dominant bacterial phyla in the waterfront area, near-shore terrestrial area, and natural area were Actinobacteria and Proteobacteria, and the dominant bacterial genus was norank_{f_*}{norank}*{o_*}{norank}*{c_*}{MB}-A2-108; the dominant fungal phylum in all three areas was Ascomycota, the dominant fungal genus in the waterfront area was unclassified*{c___}{Sordariomycetes}, while in the near-shore terrestrial area and natural area it was Preussia. (3) Mine water discharge led to significantly higher soil salinity and moisture in the desert steppe waterfront area compared with the near-shore terrestrial area and natural area; salinity inhibited the growth of soil bacteria and fungi, while simultaneously promoting the enrichment of halophilic and alkaliphilic bacteria in the study area. (4) Soil bacterial and fungal diversity and richness in the waterfront area were significantly lower than those in the natural area, further demonstrating the significant impact of mine water on soil bacterial and fungal richness and diversity; differences in richness and diversity of soil bacteria and fungi among different soil layers were relatively small; analysis of soil environmental factors and soil bacterial and fungal diversity indicated that soil salinity, water content, organic carbon, and pH were the main factors influencing desert steppe soil bacterial and fungal community diversity.

Full Text

Effects of Mine Water on Soil Microbial Community Structure and Diversity in Desert Steppe

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Abstract

To investigate the influence of mine water discharge on soil microbial community structure and diversity in desert grasslands, we studied a desert grassland site affected by mine water discharge in an arid, windy-sand region of Ningxia. High-throughput sequencing was employed to analyze bacterial and fungal community composition in soil layers (0–10 cm, 10–20 cm, and 20–30 cm) from three zones: the waterfront area along the mine water discharge lake, the near-shore terrestrial area, and a natural reference area. By integrating these data with soil physicochemical properties and other habitat factors, we identified the primary factors through which mine water disturbance affects soil microbial community structure and diversity. The results demonstrate that mine water discharge significantly altered the community composition of soil bacteria and fungi, with notable impacts on relative abundance at the phylum level. The dominant bacterial phyla across all three zones were Actinobacteria and Proteobacteria, while the predominant bacterial genus was norank_{f_*}{norank}*{o_*}{norank}*{c_*}{MB}-A2-108. Ascomycota was the dominant fungal phylum in all zones, with unclassified*{c___}{Sordariomycetes} being the main fungal genus in the waterfront area, and Preussia dominating in both the near-shore and natural areas. Mine water discharge led to significantly higher soil salinity and moisture content in the waterfront area compared to the near-shore and natural zones. While salinity inhibited the growth of soil bacteria and fungi, it simultaneously promoted the enrichment of halophilic bacteria. Both bacterial and fungal diversity and richness were significantly lower in the waterfront area than in the natural area, confirming the substantial impact of mine water on microbial richness and diversity. Differences in richness and diversity across soil layers were minimal. Analysis of soil environmental factors revealed that soil salinity, water content, organic carbon, and pH are the primary factors influencing bacterial and fungal community diversity in desert grassland soils.

Keywords: mine water; desert steppe; soil; microbial community; high-throughput sequencing

Introduction

Coal mining has significantly propelled national economic development while simultaneously causing severe ecological and environmental problems [1]. Mine water contains substantial amounts of heavy metal pollutants [2] and has become one of the primary sources of heavy metal contamination in mining areas. Characterized by high mobility and permeability, large-scale mine water discharge can alter surrounding soil physicochemical properties, exacerbate soil salinization and environmental pollution [3], and severely impair the already fragile desert grassland ecosystem' s capacity for self-repair, thereby affecting its stability and sustainable development [4]. Research indicates that mining activities can disrupt hydrological balance within and around mining areas, alter hydrogeological properties, and directly impact the ecological environment [5]. Recent studies have shown that mine water discharge affects both the structure and diversity of soil microbial communities [6], which are highly sensitive to environmental changes and serve as important biological indicators of human disturbance and ecosystem health [7].

In arid and semi-arid regions, desert grassland vegetation exhibits low diversity, precipitation is scarce with high interannual variability, and ecosystem resilience is extremely vulnerable [8]. Mine water discharge increases soil salinity in surrounding areas, changes physicochemical properties, and intensifies secondary salinization [9]. Previous studies have demonstrated that soil microbial communities respond sensitively to environmental changes and human activities [10]. Li et al. [11] investigated soil microbial succession during vegetation restoration in rare earth mine wastelands, finding that microbial community diversity and relative abundance decreased with restoration age. Peng et al. [12] studied microbial diversity in acidic mine water, revealing that mine wastewater microorganisms were dominated by typical acid mine drainage taxa, including strictly autotrophic *Acidithiobacillus* and *Leptospirillum*, as well as heterotrophic *Sulfobacillus*, *Ferroplasma*, and *Acidiphilium*. Yu [13] found that mine water discharge was a primary factor influencing spatial heterogeneity of soil properties in surrounding areas, and that changes in soil environment inevitably affected microbial community diversity. In summary, mine water discharge alters microbial community composition and reduces diversity. However, few studies have examined the intensity and spatial extent of mine water impacts on soil microbial communities, and the processes and mechanisms through which mine water affects soil microbial community structure and diversity in desert grasslands remain unclear. Investigating these mechanisms is crucial for maintaining the long-term stability of desert grassland ecosystems.

This study focuses on a desert grassland site affected by mine water discharge in an arid, windy-sand region of Ningxia, using the natural area as a control. Through high-throughput sequencing of bacterial and fungal communities in waterfront and near-shore areas, combined with analysis of soil physicochemical properties, we reveal the main factors influencing soil microbial community structure and diversity under mine water disturbance. Our findings provide a theoretical basis for effective remediation of soil pollution caused by mine water discharge and for rational utilization of mine water resources.

1.1 Study Area Overview

The study area is located in a desert grassland mine water discharge site southwest of Majiatan Town, Lingwu City, Ningxia (37°45 N, 106°44 E). Mine water analysis revealed a total nitrogen content of approximately 0.54 $\mathrm{mg \cdot L^{-1}}$, ammonia nitrogen of 0.04 $\mathrm{mg \cdot L^{-1}}$, total phosphorus of 0.52 $\mathrm{mg \cdot L^{-1}}$, total alkalinity of 169 $\mathrm{mg \cdot L^{-1}}$, and total dissolved solids of 3840 $\mathrm{mg \cdot L^{-1}}$. The region has a mid-temperate arid climate with typical continental characteristics, featuring large diurnal temperature variations and dry conditions with minimal rainfall. Soil texture consists of sandy soil and loose sandy loam with high sand content, loose structure, small water-stable aggregates, and poor water and nutrient retention capacity. Dominant vegetation includes salt- and alkali-tolerant species such as *Suaeda glauca*, *Kalidium foliatum*, *Salicornia europaea*, *Agropyron cristatum*, and *Artemisia ordosica*.

1.2 Experimental Design and Sample Collection

The sampling site was selected near the mine water discharge area without other human disturbances. Due to mine water discharge, a salinity gradient formed in the surrounding soil at varying vertical distances from the water body, affecting vegetation distribution patterns. Three zones were established: a waterfront area within 0-20 m dominated by *Agropyron cristatum*; a near-shore terrestrial area within 10-20 m dominated by *Agropyron cristatum* and *Artemisia sphaerocephala*; and a natural area within 70-100 m dominated by *Artemisia ordosica*. Three 15 m × 15 m plots were established in each zone at flat sites with uniform vegetation distribution and good growth. Within each plot, five 1 m × 1 m subplots were established using the five-point sampling method. Soil cores were collected from each subplot at depths of 0-10 cm, 10-20 cm, and 20-30 cm. Soil samples from the same depth in five subplots were mixed to form one composite sample per plot, resulting in three composite samples per depth per zone. One portion of each sample was placed in a sterile, labeled cryovial, immediately stored in a -80 °C freezer for transport, and kept at -80 °C for microbial community analysis. Another portion was placed in a ziplock bag, air-dried in the laboratory, and sieved to remove plant debris and fine roots for physicochemical analysis.

1.3 Experimental Methods

Soil water content was determined by the oven-drying method. Soil pH was measured using a PHS-3C pH meter at a soil-to-water ratio of 2.5:1. Soil organic carbon (SOC) was determined by the potassium dichromate oxidation method. Total nitrogen (TN) and total phosphorus (TP) were measured using an elemental analyzer. Available phosphorus (AP) was determined by the $NaHCO_3$ extraction-molybdenum antimony colorimetric method [14]. Soil salinity was measured by the weighing method.

For high-throughput sequencing of soil microorganisms: Soil DNA was extracted using a soil DNA extraction kit; purity and concentration were detected using a NanoDrop 2000 spectrophotometer; integrity was assessed by 1% agarose gel electrophoresis. PCR amplification was performed using primers 338F (5 -ACTCCTACGGGAGGCAGCAG-3) and 806R (5 -GGACTACHVGGGTWTCTAAT-3) for the bacterial V3–V4 region, and ITS1F (5 -CTTGGTCATTTAGAGGAAGTAA-3) and ITS2R (5 -GCTGCGTTCTTCATCGATGC-3) for the fungal ITS region. PCR products were mixed and detected by 2% agarose gel electrophoresis, then purified using an AxyPrep DNA Gel Extraction Kit. After quantification with a Quantus™ Fluorometer, samples were mixed in appropriate proportions according to sequencing requirements. Libraries were constructed using the NEXTFLEX Rapid DNA Seq Kit and sequenced on an Illumina MiSeq PE300/NovaSeq PE250 platform [15].

1.4 Data Analysis

SPSS Statistics 25 was used for one-way ANOVA of soil physicochemical indices. Mothur and Qiime were employed to generate abundance data at various taxonomic levels and to analyze alpha diversity indices (Shannon and Chao1 indices) for bacteria and fungi. One-way repeated measures ANOVA was used to compare bacterial and fungal community distributions at the phylum level among the three zones, followed by LSD tests for significant differences. Spearman correlation analysis was performed to examine correlations between environmental factors and species. Vegan package in R was used for redundancy analysis (RDA) to study the effects of mine water discharge on soil bacterial and fungal community composition. Canonical correspondence analysis (CCA) was conducted to reflect relationships between bacteria/fungi and environmental factors.

2 Results

2.1 Soil Physicochemical Properties

Mine water significantly altered soil salinity, organic carbon, total phosphorus, total nitrogen, and water content ($P < 0.05$). Soil salinity was significantly higher in the waterfront area than in the near-shore and natural areas ($P < 0.05$), while soil pH was significantly lower in the waterfront area ($P < 0.05$).

Soil organic carbon was significantly lower in the waterfront area compared to the near-shore area ($P < 0.05$). Total nitrogen and total phosphorus showed similar patterns, being significantly higher in the waterfront and natural areas than in the near-shore area ($P < 0.05$). Soil water content was significantly higher in the waterfront area than in the near-shore and natural areas ($P < 0.05$), while mine water had minimal impact on available phosphorus ($P > 0.05$).

Soil salinity, organic carbon, available phosphorus, and water content varied significantly among soil layers. Soil salinity differed significantly only in the waterfront area, showing the pattern 0-10 cm > 10-20 cm > 20-30 cm. Soil organic carbon in the near-shore and waterfront areas was significantly higher in the 10-20 cm layer than in the 20-30 cm layer. Available phosphorus decreased significantly with soil depth across all zones. Soil water content decreased with depth, while pH, total nitrogen, and total phosphorus showed minimal variation among layers [Figure 1: see original paper].

2.2 Soil Bacterial and Fungal Community Structure

Mine water discharge significantly altered soil bacterial ($P < 0.05$) and fungal ($P < 0.05$) community composition. At the phylum level, the dominant bacterial groups were Actinobacteria (33.94%), Proteobacteria (19.93%), Chloroflexi (11.93%), Acidobacteria (7.52%), Firmicutes (6.84%), Bacteroidota (6.21%), and Gemmatimonadota (5.47%). The dominant fungal phylum was Ascomycota, with an average relative abundance of 71.29%, followed by Basidiomycota (7.08%), Mortierellomycota (6.84%), Rozellomycota (3.52%), Chytridiomycota (2.47%), and Glomeromycota (1.29%). Ascomycota and Basidiomycota were the main fungal groups in the study area [FIGURE:2, FIGURE:3].

Phylum-level analysis revealed significant differences among zones. Actinobacteria relative abundance was significantly different across all three zones ($P < 0.01$), being lowest in the waterfront area and highest in the natural area. Proteobacteria abundance was significantly lower in the natural area than in the waterfront and near-shore areas ($P < 0.01$). Acidobacteria was significantly less abundant in the waterfront area than in the other two zones ($P < 0.01$). Firmicutes abundance followed the pattern: waterfront > near-shore > natural area ($P < 0.001$). Bacteroidota was significantly more abundant in the waterfront area ($P < 0.001$). Desulfobacterota was also significantly higher in the waterfront area ($P < 0.001$). Among fungi, Ascomycota was significantly less abundant in the waterfront area than in the near-shore area ($P < 0.05$). Mortierellomycota was significantly lower in the waterfront area than in the natural area ($P < 0.05$). Glomeromycota was significantly less abundant in the waterfront area ($P < 0.05$). Rozellomycota (Cryptomycota) was unique to the waterfront area [Figure 4: see original paper].

At the genus level, the dominant bacterial genera were norank_{f_}*{norank}*{o_}*{norank}*{c_}*{MB}-A2-108 (26.47%), norank*{f_}*{norank}*{o_}*{Gaiellales} (22.34%),* Bacillus

(16.13%), norank{f__}*{67}-14, norank*{f__}*{JG30}-KF-CM45,* Arthrobacter*, norank*{f__}*{norank}*{o__}*{Actinomarinales}, and norank*{f__}*{norank}*{o__}*{Vicinamibacterales}. The dominant fungal genera were* Preussia*, unclassified*{p__}*{Ascomycota}, unclassified*{c__}*{Sordariomycetes},* Mortierella*,* Metarhizium*,* Alternaria*, unclassified*{p___}{Chytridiomycota}, *Monosporascus*, and *Phaeomycocentrospora* [Figure 5: see original paper].

2.3 Soil Bacterial and Fungal Diversity Indices

Mine water discharge significantly reduced bacterial and fungal Shannon and Chao1 indices ($P < 0.05$). Bacterial Shannon index was significantly higher in the near-shore and natural areas than in the waterfront area ($P < 0.05$). Fungal Shannon index was significantly higher in the near-shore and natural areas than in the waterfront area ($P < 0.05$). Bacterial Chao1 index was significantly higher in the natural area than in the waterfront area ($P < 0.05$). Variation among soil layers was minimal, though bacterial Shannon index decreased significantly with depth in the waterfront area ($P < 0.05$), and fungal Shannon index decreased with depth in the near-shore area ($P < 0.05$) [Figure 6: see original paper].

2.4 Correlation Between Soil Environmental Factors and Microbial Phyla

Spearman correlation analysis between environmental factors and microbial community composition at the phylum level showed that soil salinity was significantly negatively correlated with Actinobacteria, Acidobacteria, Gemmatimonadota, Myxococcota, and Methylomirabilota, but significantly positively correlated with Proteobacteria, Firmicutes, Bacteroidota, and Desulfobacterota. Soil water content showed significant negative correlations with Actinobacteria, Acidobacteria, Gemmatimonadota, Myxococcota, and Methylomirabilota, and significant positive correlations with Proteobacteria, Firmicutes, Bacteroidota, and Desulfobacterota. Soil organic carbon was significantly negatively correlated with Actinobacteria, Acidobacteria, Myxococcota, and Methylomirabilota, and significantly positively correlated with Firmicutes, Bacteroidota, and Desulfobacterota. Total nitrogen and total phosphorus were significantly negatively correlated with Proteobacteria. Available phosphorus showed significant correlations with some bacterial phyla. For fungi, soil salinity was significantly negatively correlated with Basidiomycota, Mortierellomycota, Glomeromycota, Calcarisporiellomycota, and Ascomycota, but significantly positively correlated with Rozellomycota. Soil water content showed opposite patterns, being significantly positively correlated with the major fungal phyla and negatively correlated with Rozellomycota. Organic carbon was significantly positively correlated with Ascomycota and Mortierellomycota [Figure 7: see original paper].

2.5 CCA Analysis of Soil Bacteria, Fungi, and Environmental Factors

Since gradient lengths exceeded 3.0, CCA was used to analyze relationships between microbial communities and environmental factors. R^2 values represent the explanatory power of each factor. In bacterial communities, the first axis was negatively correlated with soil salinity, water content, and pH (correlation coefficients: -0.86, -0.84, -0.51), with R^2 values of 0.86, 0.84, and 0.51, respectively (all $P < 0.001$). In fungal communities, the first axis was positively correlated with soil salinity, water content, and pH (correlation coefficients: 0.89, 0.88, 0.54), with R^2 values of 0.89, 0.88, and 0.54, respectively (all $P < 0.001$). These results indicate that soil salinity, water content, and pH are the primary factors influencing soil bacterial and fungal community structure in the study area [Figure 8: see original paper].

3 Discussion

Mine water discharge significantly affected the relative abundance of bacterial and fungal phyla in the study area. The dominant bacterial phyla—Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria, Firmicutes, Gemmatimonadota, and Bacteroidota—are characteristic of saline-alkaline soils [16] and predominant in mine wastewater sediments [17]. Previous studies have identified Proteobacteria as the dominant group in saline-alkaline soils [18], though our results show Actinobacteria dominance, indicating that while bacterial groups are similar across saline environments, dominant communities vary. Actinobacteria dominance in secondary saline soils aligns with our findings [19], suggesting its important role in arid, salinized soils. The significantly lower Actinobacteria abundance in the waterfront area may relate to its preference for low water content and slightly alkaline conditions [20]. Proteobacteria and Firmicutes play important roles in sulfate reduction [21], and their enrichment in the waterfront area likely relates to sulfate degradation from mine water. Desulfobacterota was also significantly more abundant in the waterfront area, consistent with its proximity to the discharge source and high sulfate content [22]. Bacteroidota was significantly more abundant in the waterfront area, corroborating findings of high Bacteroidota content in neutral to alkaline mine water [23]. *Bacillus*, belonging to Firmicutes, exhibits strong halophilic and alkali-tolerant characteristics, enabling survival in salinized, arid environments [24]. Dominant bacterial genera such as norank_{f__*}{norank}*{o__*}{norank}*{c___}{MB}-A2-108, *Arthrobacter*, and Gaiella belong to Actinobacteria; Gaiella produces various antibiotics that facilitate survival in stressful environments [25].

The dominant fungal phyla were Ascomycota and Basidiomycota, with Ascomycota showing much higher abundance. Ascomycota’ s faster evolutionary rate and greater species diversity [26] confer strong ecological adaptability, making it the most abundant fungal group in soils [27], consistent with our findings. Saprophytic fungi decompose organic matter, with Ascomycota and Basidiomycota being primary cellulose decomposers [28], playing crucial roles in ecosystem material cycling. Rozellomycota (Cryptomycota) was unique to the waterfront area, likely due to its proximity to the discharge source and high soil moisture [29]. Among dominant fungal genera, *Preussia*, *Alternaria*, *Monosporascus*, and *Phaeomycocentrospora* belong to Ascomycota, further confirming its dominance.

Phaeomycocentrospora is halotolerant [30], and endophytic *Alternaria* can promote plant growth and enhance salt tolerance [31], contributing to stress resistance in the region' s halophytic vegetation.

Mine water significantly reduced bacterial and fungal richness and diversity, consistent with previous findings [32]. The waterfront area showed significantly lower diversity than the natural area, likely because high salinity and moisture inhibited microbial growth. Although salinity promoted enrichment of halophilic bacteria, it suppressed other bacteria and fungi [33]. The arid climate with high evaporation and low precipitation, combined with excessive moisture causing soil compaction, ultimately inhibited microbial growth, resulting in lower diversity and richness in the waterfront area.

Correlation analysis revealed that Actinobacteria was significantly negatively correlated with soil salinity but positively correlated with pH, confirming its adaptation to low-moisture, slightly alkaline, nutrient-poor conditions. Acidobacteria showed significant positive correlation with pH, contrasting with most studies but consistent with reports of Acidobacteria subgroups in neutral-alkaline environments that degrade plant residues [34]. High salinity was associated with increased relative abundance of Proteobacteria, Firmicutes, and Bacteroidota [35], which can degrade various pollutants and complex organic polymers, playing positive roles in ecological restoration. Soil salinity was significantly negatively correlated with the two dominant fungal phyla (Ascomycota and Basidiomycota) and positively correlated with Rozellomycota, indicating salt inhibition of fungi. Environmental factor analysis confirmed that soil salinity, water content, organic carbon, and pH are the main factors influencing microbial community diversity.

4 Conclusions

1) Mine water discharge significantly altered soil bacterial and fungal community composition in the study area, particularly affecting relative abundance at the phylum level.
2) Salinity inhibited bacterial and fungal growth in the waterfront area while promoting enrichment of halophilic bacteria.
3) Both bacterial and fungal richness and diversity were significantly lower in the waterfront area than in the natural area, confirming the substantial impact of mine water on microbial diversity.
4) Soil salinity, water content, organic carbon, and pH are the primary factors influencing soil bacterial and fungal community diversity in the study area.

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Note: Figure translations are in progress. See original paper for figures.