



Article

Changes in Nutrient-Regulated Soil Microbial Communities in Soils Concomitant with Grassland Restoration in the Alpine Mining Region of the Qilian Mountains

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Abstract: In response to the significant ecological damage caused by unsustainable mining practices in the Qilian Mountains, ecological restoration projects have been undertaken in recent years. Analyzing the changes in soil microbial communities during the restoration process of mine meadows helps to reveal the mechanism of the restoration process in alpine mining areas. To explore the characteristics of soil microbial community distribution and their relationships with soil environmental factors during the restoration of alpine grasslands in the Qilian Mountains, we conducted surveys and analyses in two restoration levels low restoration (LR) and high restoration (HR) in the eastern Qilian Mountains, along with an undisturbed natural grassland control (NG). We found that as the degree of high-altitude mining area recovery increases, there were significant increases in vegetation cover, vegetation height, above-ground biomass, vegetation Shannon–Wiener index, soil organic carbon (SOC), soil water content (SWC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), available phosphorus (AP), and available nitrogen (AN). Conversely, soil pH and electrical conductivity (EC) significantly decreased, with soil pH decreasing from 6.93 to 4.13. Restoration of high-altitude mining area grasslands significantly alters the distribution and composition of soil bacteria and fungi, while the impact on soil microbial community changes was not significant. Notably, with increasing recovery level, the dominant bacterial phyla are Acidobacteria and Proteobacteria, while the dominant fungal phyla are Ascomycota and Basidiomycota. These results indicate that changes in vegetation and soil properties both affect the composition of soil microbial communities, with soil properties having a greater influence. Soil fertility and nutrient levels emerge as the primary drivers influencing soil microbial composition communities and the degree of high-altitude mining area grassland recovery.

Keywords: soil microbial composition; alpine grassland; mining area; vegetation characteristics; soil physicochemical properties



Citation: Yang, X.; Feng, Q.; Zhu, M.; Yang, L.; Zhang, C.; Zhang, J.; Wang, Z.; Feng, Y. Changes in Nutrient-Regulated Soil Microbial Communities in Soils Concomitant with Grassland Restoration in the Alpine Mining Region of the Qilian Mountains. *Agronomy* **2023**, *13*, 3052. <https://doi.org/10.3390/agronomy13123052>

Academic Editor: Sergio Molinari

Received: 25 October 2023

Revised: 4 December 2023

Accepted: 11 December 2023

Published: 13 December 2023



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1. Introduction

The alpine grassland ecosystem is one of the most critical ecosystems globally and serves as the primary ecosystem in the Qilian Mountains [1,2]. It encompasses a wide variety of soil types and soil microorganism species. Furthermore, it serves as the material foundation for pastoralism and agricultural production by local farmers and herders [3]. Additionally, it serves as the headwaters of inland river basins such as the Heihe River Basin and Shiyang River Basin in the northwest China region, as well as a water source for

the Hexi Oasis region. The mining industry plays a significant role in the Qilian Mountains region and contributes to the economic advancement and industrial development of the northwest China region through the exploration and utilization of mineral resources [4,5]. However, mining activities in this area have led to a range of ecological environmental issues, including soil erosion, decreased soil quality, vegetation destruction, and alterations in soil and soil microorganism species diversity, particularly in the alpine grasslands [6]. In the early stages of mining operations in the Qilian Mountains, extensive open-pit mining activities resulted in severe damage to the surrounding grassland ecosystems and soil, leading to numerous ecological environmental problems [7,8].

Since the 1950s, there has been a lack of proper planning in mining activities, leading to a disregard for the protection of the ecological environment in mining areas [9]. This has been compounded by various factors such as mining conditions, extraction methods, production processes, and technological equipment, resulting in a series of severe ecological environmental issues that have caused significant damage to the ecological environment of the Qilian Mountains [10]. The Qilian Mountains are a vital component of China's western ecological security barrier and serve as the primary ecological region for the development of ecological protection and restoration efforts in the Qilian Mountains' landscape of mountains, rivers, forests, fields, lakes, and wetlands [11,12]. Their significance lies in effectively improving the ecological environment of the Hexi Corridor and building a Western ecological security barrier for China [13,14]. The ecological environmental restoration in the high-altitude mining areas of the Qilian Mountains mainly includes measures related to mining site environmental remediation, land reclamation and pollution remediation, biodiversity conservation, and ecological management in key areas [15]. In recent years, environmental remediation efforts in the Qilian Mountains mining areas have primarily focused on artificial restoration and natural recovery [16]. Under the guidance of natural recovery as the primary restoration method, significant progress has been made in ecological restoration. The study of soil characteristics and soil microbial diversity is a crucial aspect of current research in community ecology during ecological restoration [17,18]. Analyzing the changes in plant species diversity and soil microbial diversity resulting from different restoration methods in mining areas is of great significance for evaluating ecological restoration theories in high-altitude mining areas and conducting ecological restoration and reconstruction in mining areas [19].

Soil serves as the nutrient foundation for ecosystem productivity, and competition among plants and species can lead to changes and successions in the ecological environment [20]. During the process of changes and successions in plant communities, the physicochemical properties of soil can directly impact the recovery process and growth conditions of grassland vegetation [21]. Different levels of restoration exhibit variations in soil physicochemical properties. Soil microorganisms are a crucial component of soil ecosystems, primarily involved in regulating the cycling of soil organic matter and nutrient elements, serving as a reservoir of effective nutrients for plants. Soil microorganisms play a key role in driving ecosystem cycles and are central to the decomposition of soil organic matter and nutrient cycling [22,23]. Soil microorganisms are sensitive to environmental changes, and alterations in soil temperature, humidity, physical properties, chemical properties, and vegetation can all induce changes in soil microbial communities; since the restoration of alpine grassland in the Qilian Mountains' cold mining area, there has been a noticeable increase in vegetation. Further research is needed to explore soil fertility and microbial composition [24,25].

The degradation of alpine mining grasslands due to mining activities can significantly impact soil microorganisms [26]. Firstly, the degradation of alpine mining grasslands leads to a reduction in primary ecosystem productivity and vegetation cover. This alteration in the composition of grassland plant communities indirectly affects the distribution and composition of soil microorganisms [27]. Secondly, changes in the composition of plant communities directly influence the occurrence of plant-related pathogens [28]. Thirdly, the degradation of alpine mining grasslands reduces the quantity of soil organic carbon

(SOC), thereby inhibiting the proliferation of soil microorganisms and reducing their population [29]. Fourthly, the degradation of alpine mining grasslands decreases soil moisture levels, reducing the activity of soil microorganisms and altering the composition of soil microbial communities. Additionally, the mining process byproducts (slag) must add calcium carbonate, gypsum, or similar minerals to the soil material; and the restoration of alpine mining grasslands can reduce soil pH, impacting the composition of soil microbial communities [30]. In summary, soil microorganisms are closely linked to changes in vegetation and soil properties during the degradation and restoration processes of alpine grasslands. However, research on the varying impacts of soil microorganisms on the restoration of alpine mining grasslands is limited. A comprehensive understanding of the effects of alpine mining grassland restoration on soil microorganisms will aid in evaluating the outcomes of alpine mining grassland of the Qilian Mountains restoration and contribute to our understanding of mining area grasslands, as well as predicting the management and restoration of grassland ecosystems in high-altitude mining areas of the Qilian Mountains [31].

The main objective of this study was to elucidate the variations in soil physicochemical properties and soil microbial community dynamics during mining area grassland restoration. Additionally, we aimed to understand the regulatory mechanisms of these changes and the factors influencing soil microbial community dynamics during the restoration process of alpine mining area grasslands [32,33]. In our study, we investigated the impact of alpine mining area grassland restoration on soil microbial communities and the influence of different restoration levels on these communities in the low-restoration (LR), high-restoration (HR), and natural-grassland (NG) alpine meadows in the Qilian Mountains. We proposed that (i) changes in the vegetation characteristics and soil physicochemical properties of the mining area grassland directly impact the composition of soil microbial communities. (ii) As the restoration degree of the mining area grassland increases, the composition characteristics of soil microbial communities become more complex. (iii) Among different restoration levels in mining area grasslands, vegetation characteristics had a more significant influence on soil microbial community composition compared to soil physicochemical properties. We validated these hypotheses by assessing the variations among vegetation, soil properties, and soil microbial communities at different restoration levels in alpine mining area grasslands and analyzing the relationships among vegetation, soil properties, and soil microbial communities.

2. Material and Methods

2.1. Study Site, Experimental Design, and Soil Sampling

The study was conducted in the alpine mining grasslands of the Qilian Mountains, specifically in the Jinqianghe area, Zhuaxixiulong Town, Tianzhu County, Gansu Province, China. This region, situated at the eastern end of the Qilian Mountains' northern slope in Gansu Province, features high elevation, complex topography, and a continental high-cold semi-arid climate. It encompasses 301,200 acres of grasslands and 172,000 acres of forests, with an average elevation of 3000 m [34]. The region is resource-rich, hosting coal and precious metals such as gold, lead, zinc, manganese, and iron. Gold mining, in particular, is a cornerstone of the local economy. The region experiences an annual average temperature of $-0.1\text{ }^{\circ}\text{C}$, with an average annual evaporation of 1590 mm and annual precipitation ranging from 400 to 500 mm, the soil is dominated by subalpine meadow soil and subalpine black calcium soil.

The degradation and restoration of alpine mining grasslands in this high-cold mining area are prolonged processes that defy concise quantification within a short timeframe. Hence, a temporal scale is employed to represent the restoration process, as opposed to a spatial one. Long-term underground mining at the Jinqianghe Gold Mine, without adequate measures for protecting the grassland ecological environment, has caused severe damage to soil and vegetation, resulting in the accumulation of mine slag heaps that have adversely affected the ecological environment and caused issues such as soil erosion [35].

Before mining activities, vegetation cover exceeded 80%, but after mining, the aboveground vegetation biomass significantly declined, leaving extensive barren areas. The method of restoring succession sequences is based on the “Soil erosion classification and grading standard” (SL190-2007) of China. By the coverage of natural restoration removing debris and sowing seeds recovery begun in 2018, the restored alpine mining grasslands were classified based on their aboveground vegetation cover into low restoration level (LR), high restoration level (HR), and natural grassland (NG): among them, natural grassland (NG) as CK. This study comprises three sampling points, with 15 replicates set for each, soil samples were collected at a 0–20 cm depth, resulting in a total of 45 soil samples. Of these, 45 soil samples were utilized for soil microbial analysis. In each restoration level site, five 50 cm × 50 cm quadrats were randomly selected in different directions (Figure 1). Similar average elevation and annual precipitation were recorded for similar sites. The prevailing soil type in the study area is alpine meadow soil, and specific site information is detailed in Table 1.

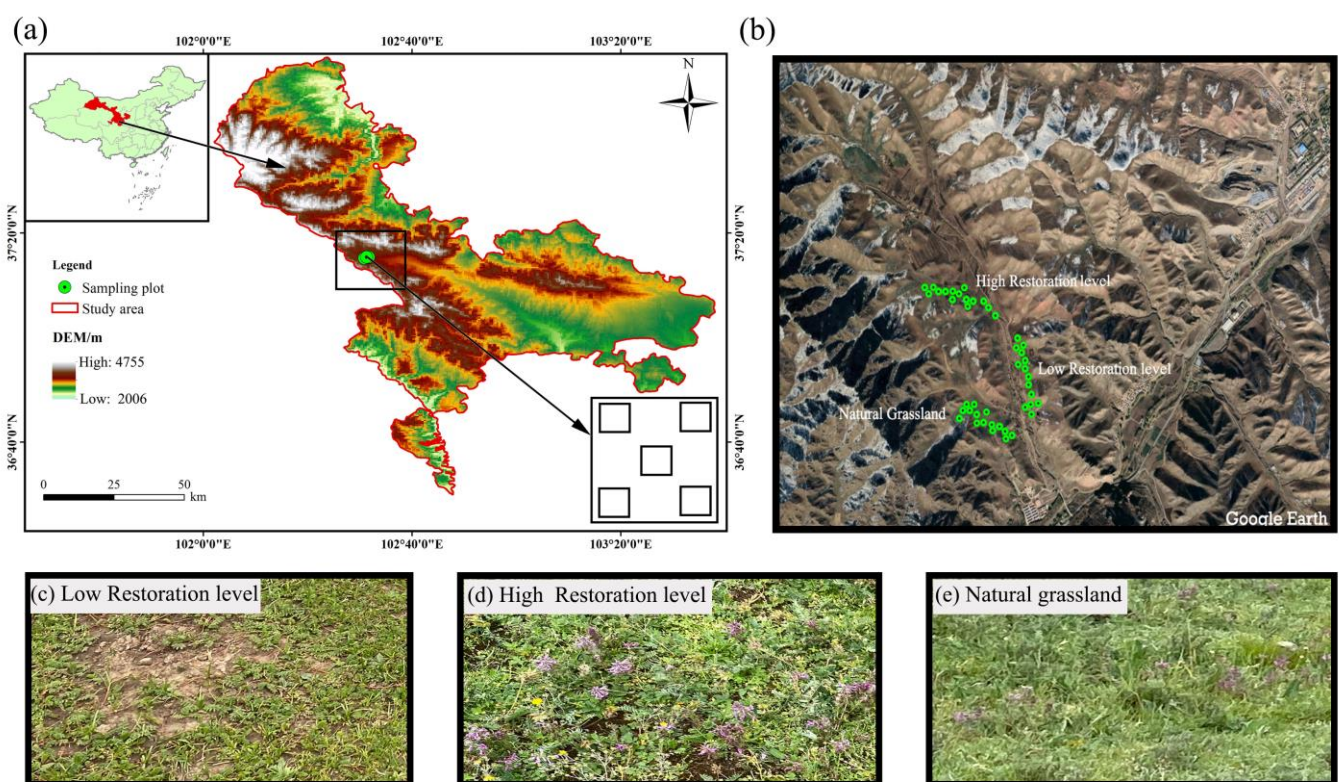


Figure 1. Location of the study area on the Chinese Qilian Mountains (a,b), LR, low-restoration level (c), HR, high-restoration level (d), and NG, CK natural grassland (e).

Table 1. Basic information of different restoration levels in the alpine mining grassland.

Recovered Degree	Geography Coordinate	Altitude/m	Above-Ground Biomass (g m^{-2})	Vegetation Cover
Low restoration level (LR)	37°25′67″ N 102°52′24″ E	3294	130.73 ± 5.64 c	0.25 ± 0.15 b
High restoration level (HR)	37°25′61″ N 102°52′82″ E	3292	360.85 ± 2.74 b	0.77 ± 0.03 a
Natural grassland (NG)	37°25′62″ N 102°52′18″ E	3295	426.36 ± 9.05 a	0.88 ± 0.05 a

Note: Different letters on the back of the values between treatments indicate significant differences at the 0.05 level.

This study was conducted in August 2022, with measurements of plant height, plant density, vegetation cover, and plant community composition taken in the field for each plot [22]. All aboveground plants in quadrats were clipped, dried at 65 °C for 24 h, and then weighed to determine aboveground biomass. Soil samples were collected randomly from

each plot at depths of 0–20 cm using a 5 cm-diameter soil auger. The samples were mixed, sieved through a 0.22 cm sieve to remove impurities, placed in sterile, sealed bags, stored at low temperatures, and transported to the laboratory for the analysis of soil physicochemical properties. A portion of the soil samples was used for soil physicochemical analysis, while another portion was stored at $-80\text{ }^{\circ}\text{C}$ for subsequent analysis of soil microbial communities.

2.2. Soil of Physical and Chemical Properties

Soil pH was measured using a pH meter. Soil electrical conductivity (EC) was determined by mixing soil and distilled water at a 1:2.5 ratio and measuring with a conductivity meter. Soil water content (SWC) was calculated by weighing fresh soil samples, then drying them at $105\text{ }^{\circ}\text{C}$ for 48 h, and calculating the difference in weight to determine the moisture content [36]. Soil total organic carbon (SOC) was analyzed using the potassium dichromate digestion method with external heating [36]. Soil total phosphorus (TP) content was measured by digesting samples with H_2SO_4 and HClO_4 , followed by UV spectrophotometric analysis (UV2800 Spectrophotometer, Shanghai, China) [36]. Soil total potassium (TK) was determined using flame photometry. Soil total nitrogen (TN) and ammonium nitrogen (AN) were quantified using the Kjeldahl method and alkaline diffusion method, respectively [37]. Soil available phosphorus (AP) was assessed using the molybdenum blue colorimetric method [38].

2.3. Soil DNA Extraction, PCR and Sequencing

The total DNA from soil samples was extracted with E.Z.N.A Soil DNA Kits (Omega, Norcross, GA, USA) and determined by 1% agarose gel electrophoresis for detection. The primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the V3–V4 region of the 16S rRNA gene [37]. The PCR program was run at $95\text{ }^{\circ}\text{C}$ for 5 min, followed by 25 cycles of $94\text{ }^{\circ}\text{C}$ for 45 s, $50\text{ }^{\circ}\text{C}$ for 30 s and $72\text{ }^{\circ}\text{C}$ for 30 s, and a final extension at $72\text{ }^{\circ}\text{C}$ for 6 min.

The fungal PCR primers ITS5F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') were used to amplify the ITS1 region of the ITS rRNA gene [38]. The PCR program was run at $95\text{ }^{\circ}\text{C}$ for 5 min, followed by 28 cycles of $95\text{ }^{\circ}\text{C}$ for 30 s, $55\text{ }^{\circ}\text{C}$ for 30 s and $72\text{ }^{\circ}\text{C}$ for 45 s, and a final extension at $72\text{ }^{\circ}\text{C}$ for 5 min. The fungal PCR products were determined by 1% agarose gel electrophoresis for detection. PCR was performed with 20 μL of the reaction mixture, which included 5 μL of $2 \times \text{Taq PCR Master Mix}$ (Shanghai Sangong Biotechnology Co., Ltd., Shanghai, China), 1 μL of each primer (10 μM), 1 μL of total DNA (10 ng), and sterilized ultrapure water. They were purified by the SanPrep Column DNA Gel Extraction Kit (Shanghai Sangon Biotechnology Co., Ltd., Shanghai, China) and quantified by QuantiFluorTM-ST (Promega, Madison, WI, USA). The purified amplicons were sequenced using the Illumina MiSeq platform sequencing (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China, <http://www.majorbio.com/> accessed on May 2022).

2.4. Soil Bioinformatics Analysis

The paired-end reads originating from the original DNA fragments were merged using FLASH 1.2.11 software, followed by quality filtering using QIIME 1.9.1 software. Only effective sequences were kept, and unassembled reads were excluded. Unique sequences sharing a similarity of 97% or more were clustered into operational taxonomic units (OTUs) using UPARSE 7.0.1 software. Subsequently, each OTU was annotated through the SSU-rRNA SILVA database using MOTHUR 1.30.2 [39].

For data normalization, we used the samples with the least available data as the reference. Soil microbial community diversity and richness were evaluated using QIIME 1.7.0 software. In total, we obtained 3,898,663 high-quality, chimera-free 16S rRNA gene sequences and 3,913,731 ITS gene sequences from all soil samples. The 16S rRNA sequences per sample ranged from 67,265 to 173,430, while the fungal ITS sequences per sample ranged from 50,499 to 138,244. Good's coverage for bacterial and fungal commu-

nities varied from 98.6% to 99.2% and 99.0% to 99.9%, respectively. Across all samples, 3672 bacterial OTUs and 3073 fungal OTUs were identified, with 2276 bacterial OTUs and 423 fungal OTUs shared among all samples.

2.5. Statistical Analysis

One-way analysis of variance (ANOVA) was assessed through SPSS 20.0 software to detect significant differences of the vegetation and soil properties, with statistical significance determined by Duncan tests ($p < 0.05$). Rarefaction curves and a Venn diagram depicting shared and unique operational taxonomic units (OTUs) were generated using MOTHUR v.1.33.3 software. Principal coordinate analysis (PCoA) and an analysis of similarities (ANOSIM) were conducted to determine the bacterial and fungal community relationship differences by a Bray–Curtis distance in community compositions and Adonis is the function in R. The identification of significantly distinct bacterial and fungal communities across different restoration levels was carried out using the linear discriminant analysis effect size (LEfSe) method. For the analysis and prediction of bacterial and fungal community functions, FAPROTAX (<http://www.zoology.ubc.ca/louca/FAPROTAX> accessed on September 2023) and FUNGuild (<http://www.funguild.org/>) were employed, respectively. Redundancy analysis (RDA) was conducted to examine the relationship between soil microbial community composition and environmental factors, with the model's significance assessed using Monte Carlo permutation (999 iterations). The correlation between the relative abundance of soil bacterial and fungal communities and environmental factors was assessed using the Spearman correlation matrix. Variance partitioning analysis (VPA) was conducted to evaluate the relative impact of plant and soil properties on soil microbial community composition, utilizing the “varpart” function in the vegan package within R version 4.2.3 [40].

3. Results

3.1. Changes in Vegetation Characteristics and Soil Properties across Different Restoration Levels

With increasing alpine grassland restoration, vegetation cover, height, and the Shannon–Wiener index all significantly increased (Table 2). Soil parameters, including soil water content (SWC), soil organic carbon (SOC), ammonium nitrogen (AN), available phosphorus (AP), total potassium (TK), total nitrogen (TN), and total phosphorus (TP), all showed significant increases ($p < 0.001$). Additionally, as restoration levels increased, soil pH and electrical conductivity (EC) significantly decreased ($p < 0.001$). When comparing high restoration (HR) levels to natural grassland, the only notable difference was in soil EC, which showed no significant variation (Figure 2).

Table 2. Vegetation properties of different restoration levels in the alpine mining grassland.

Parameters	LR	HR	NG	<i>p</i>
Density (n m^{-2})	269.10 $\pm$ 45.49 c	377.33 $\pm$ 11.55 b	385.16 $\pm$ 17.63 a	0.354
Height (cm)	7.79 $\pm$ 0.49 b	12.96 $\pm$ 4.04 a	15.24 $\pm$ 0.17 a	<0.001
Shannon–Wiener index	1.69 $\pm$ 0.33 b	2.20 $\pm$ 0.23 a	2.32 $\pm$ 0.29 a	0.005

Note: Distinct lowercase letters within the same row denote statistically significant differences ($p < 0.05$) among various restoration levels. LR, low restoration level; HR, high restoration level; NG, natural grassland.

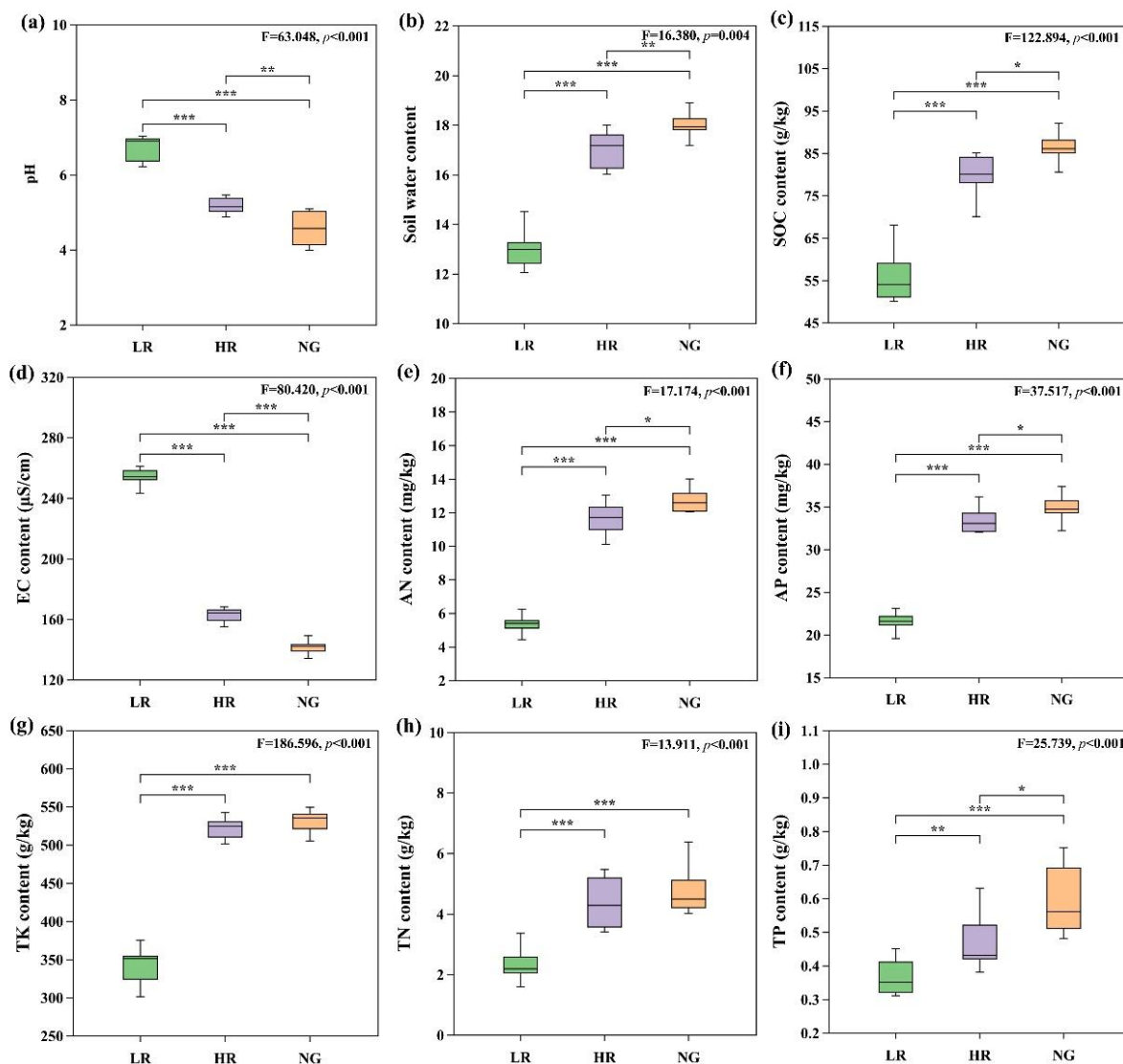


Figure 2. Soil properties of different restoration levels in the alpine mining grassland. LR, low restoration level; HR, high restoration level; NG, natural grassland; SWC, soil water content; SOC, soil organic carbon; EC, electrical conductivity; AN, available nitrogen; AP, available phosphorus; TK, total potassium; TN, total nitrogen; TP, total phosphorus. (a) pH; (b) SWC, soil water content; (c) SOC content; (d) EC content; (e) AN content; (f) AP content; (g) TK content; (h) TN content; (i) TP content. * indicates significant correlation ($p < 0.05$), ** indicates extremely significant correlation ($p < 0.01$), *** indicates extremely significant correlation ($p < 0.001$).

3.2. Soil Microbial Community Composition for Grassland Restoration in Alpine Mining Areas

From a total of 45 samples, we acquired 3,898,663 bacterial amplicon sequences, each with an average length of 416 bp, and 3,913,731 fungal amplicon sequences, each averaging 246 bp. These sequences were subsequently grouped into 36,569 bacterial operational taxonomic units (OTUs) and 6546 fungal OTUs. Soil bacteria contained 46 phyla, 155 classes, 399 orders, 653 families, 1319 genera, and 3487 species. There were 2764 of the same OTUs in the LR, HR, and NG restoration of mine site grasslands, and there were 963 of the same OTUs in the HR and NG restoration of mine site grasslands. Soil fungi contained 18 phyla, 64 classes, 145 orders, 329 families, 757 genera, and 1284 species. There were 305 of the same OTUs in the LR, HR, and NG restoration of mine site grasslands, and there were 143 of the same OTUs in the HR and NG restoration of mine site grasslands (Figure 3).

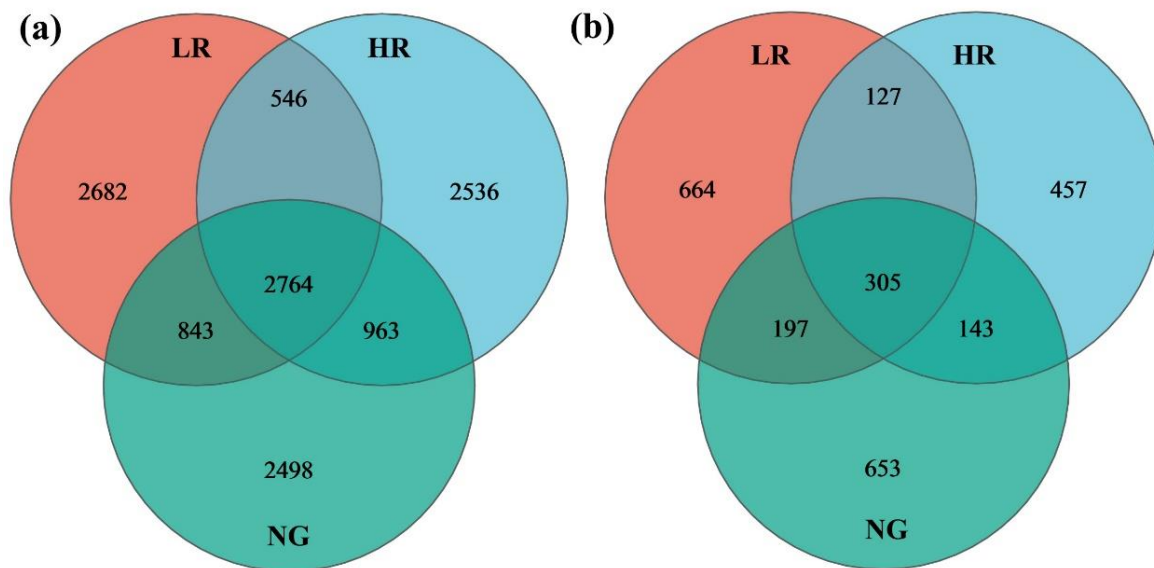


Figure 3. Number of distinct and shared bacterial (a) and fungal (b) OTUs among LR grassland, HR grassland, and NG grassland, LR, low restoration level; HR, high restoration level; NG, natural grassland.

The top five bacterial phyla observed were Actinobacteriota, Proteobacteria, Acidobacteriota, Chloroflexi, and Gemmatimonadota (Figure 4a). As the degree of grassland restoration increases in alpine mining areas, the relative abundance of Actinobacteriota and Proteobacteria initially increases, followed by a decline. In contrast, the relative abundance of Acidobacteria, Gemmatimonadota, Bacteroidota, and Patescibacteria initially decreased then increased. Meanwhile, the relative abundance of Methyloirabillota, Myxococcota, and unclassified_k_norank_d_Bacteria showed a gradual increase, with significant variations observed across different restoration levels ($p < 0.05$) (Figure 4a). At the genus level within the bacterial community, the degree of grassland restoration produces an initial increase and subsequent decrease in the relative abundance of Streptomyces, norank_f_67-14, Bradyrhizobium, Sphingomonas, and norank_f_JG30-KF-CM45. Conversely, the relative abundance of norank_f_norank_o_Vicinamibacterales, Bacillus, norank_f_Gemmatimonadaceae, Sphingomonas, and Pseudonocardia exhibited an initial decrease followed by an increase (Figure 4b).

The top five fungal phyla observed were Ascomycota, Mortierellomycota, Basidiomycota, unclassified_k_Fungi, and Chytridiomycota (Figure 4c). As the degree of grassland restoration increased in alpine mining areas, the relative abundance of Ascomycota and Basidiomycota initially increased, followed by a decline. In contrast, the relative abundance of Mortierellomycota and unclassified_k_Fungi initially decreased then increased. Meanwhile, the relative abundance of Rozellomycota, Chytridiomycota, Olpidiomyota, and Glomeromycota decreased gradually, with significant variations observed across different restoration levels ($p < 0.05$) (Figure 4c). At the genus level within the fungal community, the degree of grassland restoration produces an initial increase and subsequent decrease in the relative abundance of Fusicolla and Dactylonectria. Conversely, the relative abundance of Mortierella exhibited an initial decrease followed by an increase. Meanwhile, the relative abundance of Dactylonectria, unclassified_k_Fungi, Cistella, Cadophora, and unclassified_o_Helotiales decreased gradually with different restoration levels (Figure 4d).

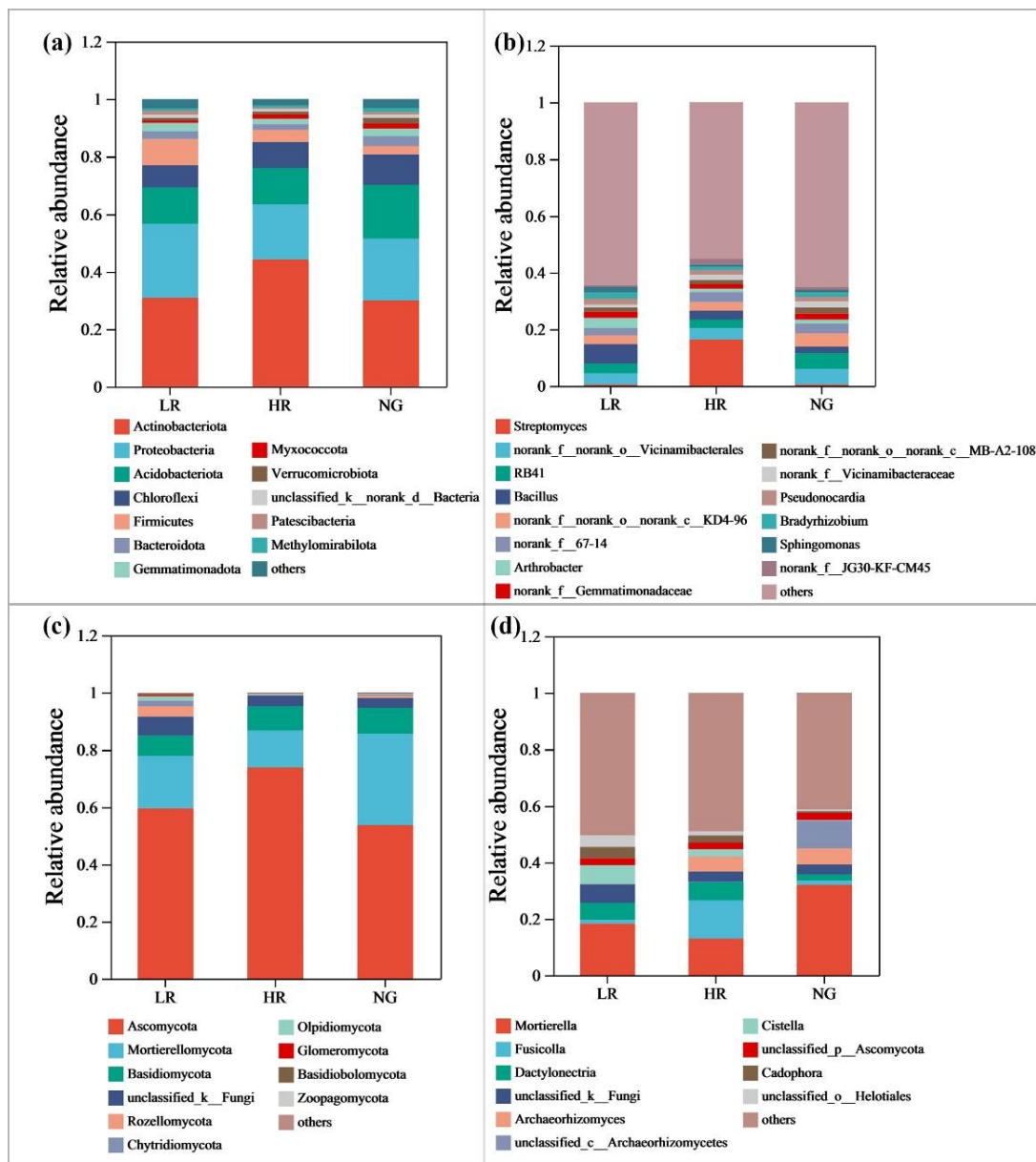


Figure 4. The relative abundance of predominant bacterial communities (a,b) and fungal communities (c,d) at the phylum and genus levels in alpine mining grassland with varying restoration levels. LR, low restoration level; HR, high restoration level; NG, natural grassland.

3.3. Soil Microbial Community Alpha and Beta Diversity for Grassland Restoration in Alpine Mining Areas

Concerning bacterial communities, the diversity indices (Shannon–Wiener and Simpson) exhibited their highest values in NG and the lowest in LR, whereas the richness indices (Chao1 and ACE) reached their peaks in HR and their troughs in LR (Figure 5). For bacteria communities, there were significantly greater levels than at the OTU level in either the Shannon or Simpson index between HR and NG (Figure 5). However, the fungal Shannon index for HR was significantly greater than NG (Figure 5). Principal coordinate analysis (PCoA) was employed to compare the β -diversity of bacteria and fungi across three recovery levels. For bacteria, based on the Bray–Curtis distance in PCoA, the bacterial communities produced three distinct clusters corresponding to the three restoration levels of alpine meadows (ANOSIM: $R = 0.136$, $p = 0.085$; Adonis: $R^2 = 0.262$, $p = 0.045$) (Figure 6a). Soil

fungus communities showed distinct clusters between HR and NG grassland restoration levels (ANOSIM: $R = 0.161$, $p = 0.078$; Adonis: $R^2 = 0.324$, $p = 0.020$) (Figure 6b).

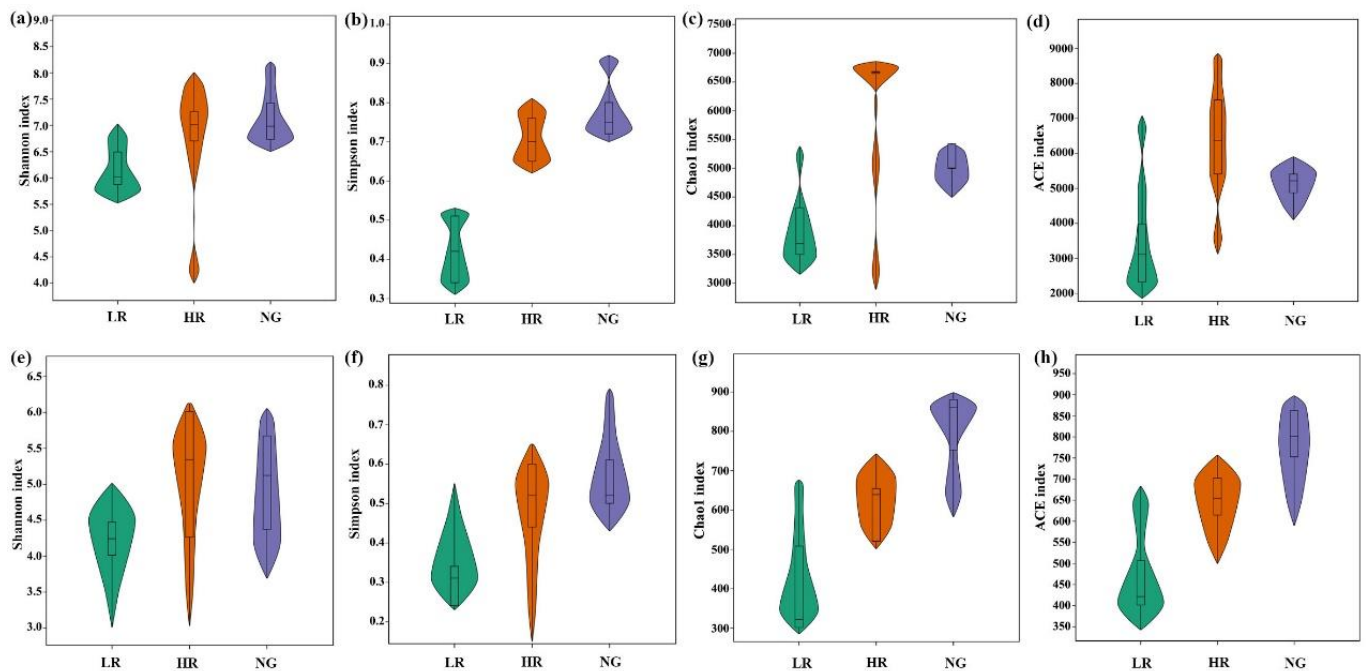


Figure 5. The soil bacterial and fungal diversity and richness indices in alpine mining grassland with varying restoration levels are described by (a–d) representing the Shannon, Simpson, Chao 1, and ACE indices of bacterial communities, respectively. Additionally, (e–h) represent the Shannon, Simpson, Chao 1, and ACE indices of fungal communities, respectively. LR, low restoration level; HR, high restoration level; NG, natural grassland.

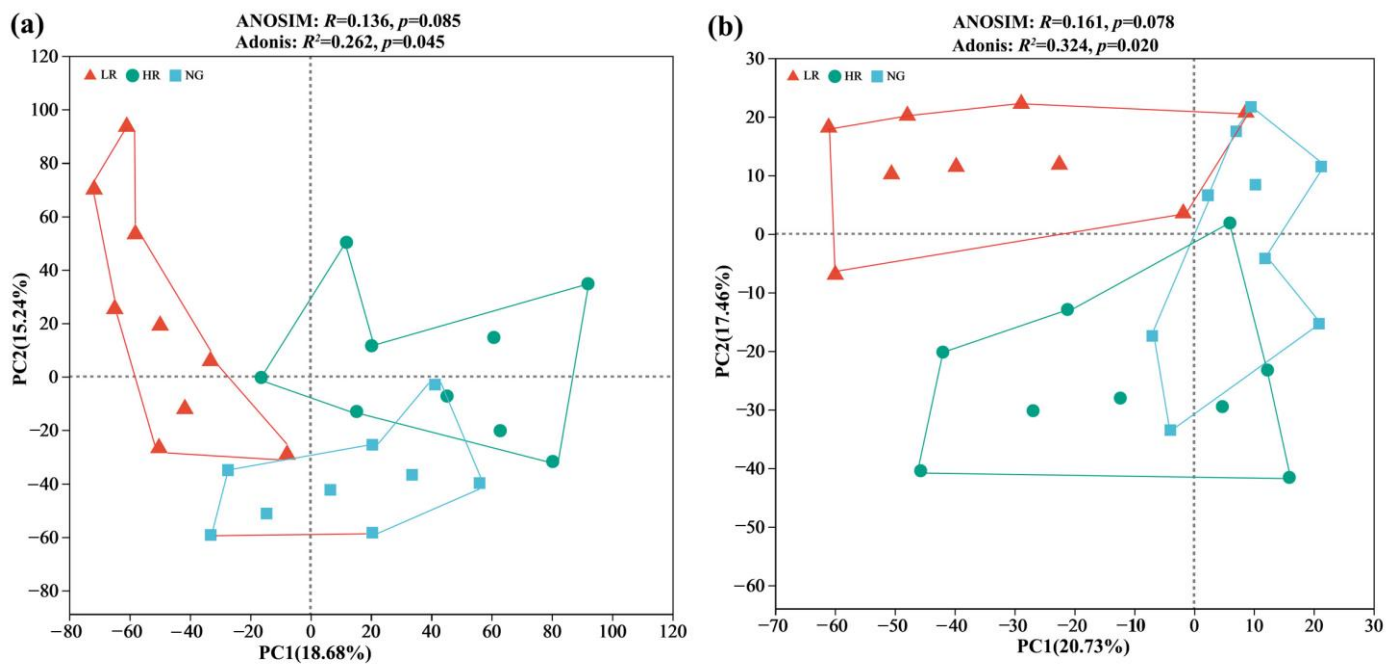


Figure 6. Principal coordinate analysis (PCoA) of bacterial communities (a) and fungal communities (b) in alpine mining grassland with varying restoration levels. LR, low restoration level; HR, high restoration level; NG, natural grassland.

The compositions of bacterial and fungal communities exhibited significant differences across the restoration levels, as indicated by the LefSe analysis (Figure 7). In the LR, substantial enrichment was observed in Norank (phyla and its genus SC-I-84), Caulobacteraceae (from family to genus), Nakamurellaceae (family), Oxalobacteraceae (from family to species), Bradyrhizobium (genus), and Massilia (from genus to species). HR showed enrichment in Tremellaceae (family), Trichomeriaceae (family), and Gibberella (genus). In the NG, substantial enrichment was observed in Thelephorales (from order to genus), Thelephoraceae (from family to species), Aspergillus (genus), and Tomentella (genus) (Figure 7).

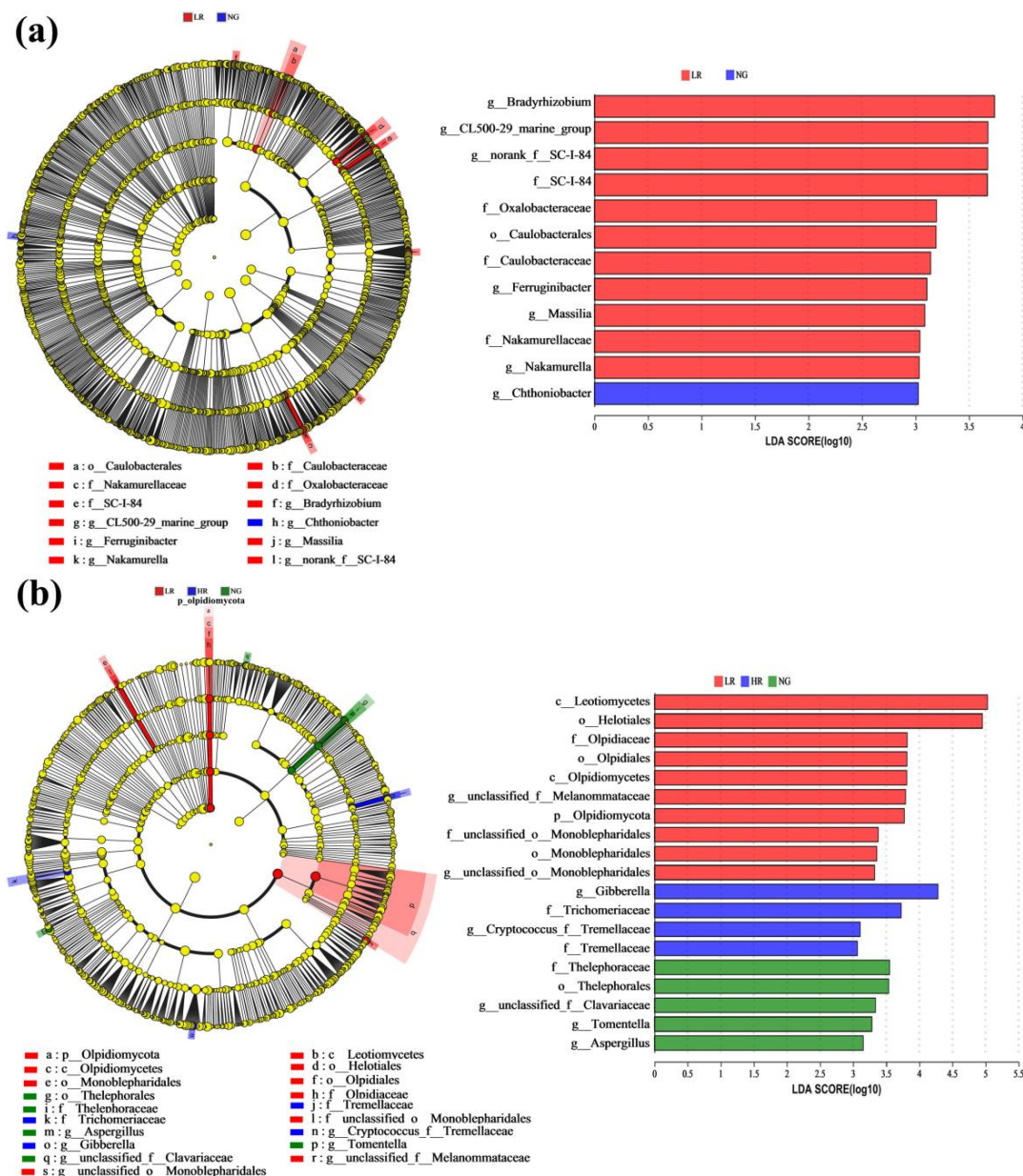


Figure 7. Cladogram illustrating the phylogenetic distribution of bacterial lineages (a) and fungal lineages (b) in alpine mining grassland with different restoration levels. The histogram of linear discriminant analysis (LDA) scores was computed for species showing differential abundance in bacterial (a) and fungal (b) communities in alpine mining grassland with varying restoration levels, identified using a threshold value of 3.0. LR, low restoration level; HR, high restoration level; NG, natural grassland.

3.4. Functional Profiles of Soil Microorganisms for Grassland Restoration in Alpine Mining Areas

The analysis of soil bacterial community function indicated the principal functional categories within each plot encompassed “Amino acid transport and metabolism”, “Energy production and conversion”, and “Transcription”. The soil bacterial function had no difference at the OTU level with the three restoration grasslands (Figure 8a). Soil fungal community functional predictions derived from FUNGuild results indicated the primary functional categories as “Undefined Saprotroph”, “Endophyte”, “Wood Saprotroph”, and “Plant Pathogen”. In response to alpine meadow restoration, “Undefined Saprotroph” and “Plant Pathogen” exhibited an initial increase followed by a decrease, while “Endophyte-Lieeer Saprotroph-Soil Saprotroph-Undefined Saprotroph” displayed the opposite trend (Figure 8b). In comparison to LR, HR witnessed a 30.51% increase in the relative levels of “Undefined Saprotroph” and a 33.57% increase in “Plant Pathogen”, Conversely, NG experienced a 13.57% decrease in “Undefined Saprotroph” and a 38.26% decrease in “Plant Pathogen” (Figure 8b).

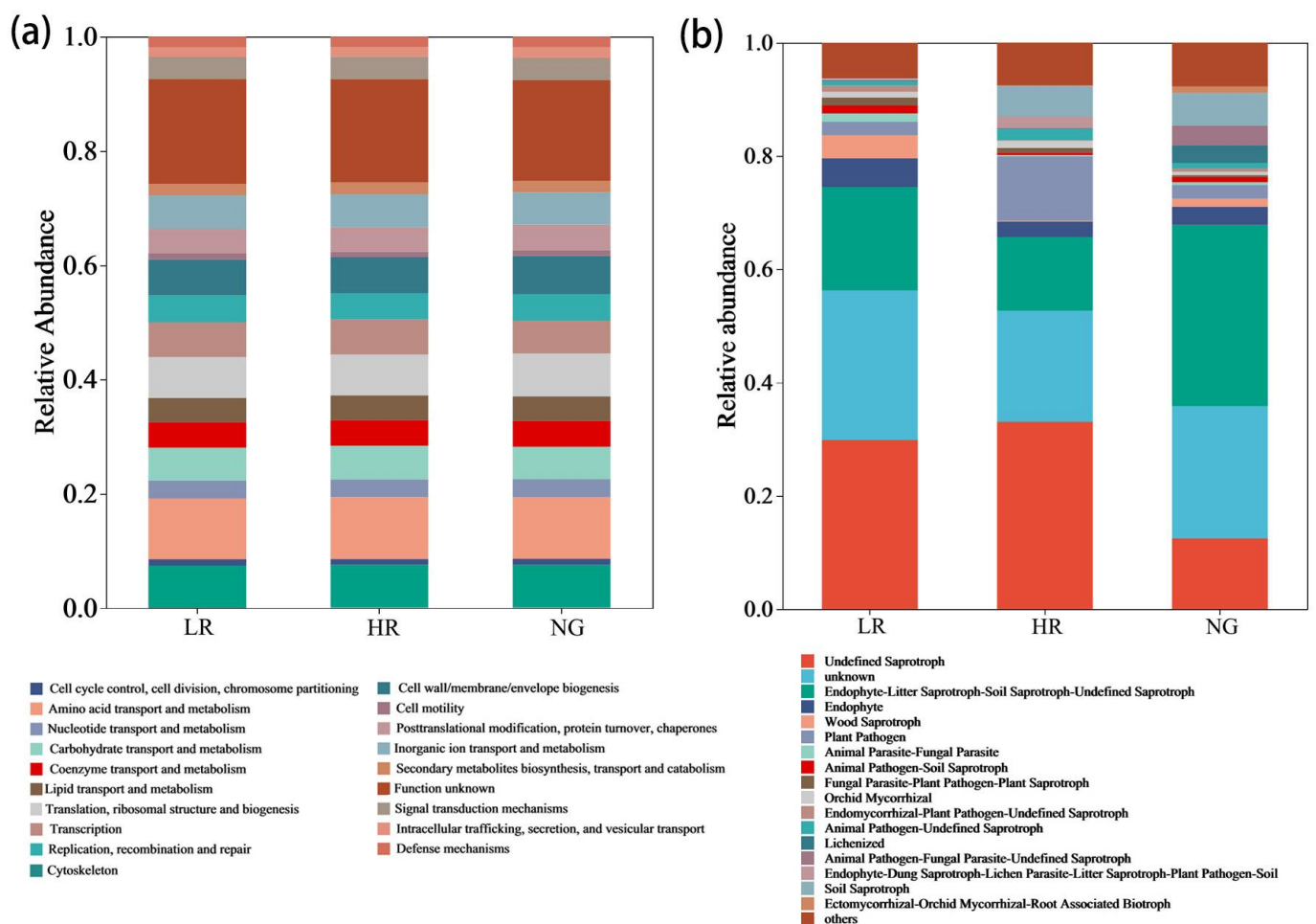


Figure 8. Predicted functional profiles of soil bacteria (a) and fungi (b) in alpine mining grassland with varying restoration levels. LR, low restoration level; HR, high restoration level; NG, natural grassland.

3.5. The Correlation between Soil Microbial Community and Environmental Factors for Grassland Restoration in Alpine Mining Areas

The relationships between soil microbial communities and environmental variables at various restoration levels are illustrated in Figure 9. Redundancy analysis (RDA) was employed to identify the primary influencing factors and trends. For bacterial community structure RDA analysis, RDA1 explained 86.84% of the variance, while RDA2 explained 6.56%. Notably, SWC, SOC, AP, AN, TK, TN, and TP were the major influencing factors on bacterial community structure (Figure 9a). Regarding fungal community structure

RDA analysis, RDA1 explained 44.95% of the variance, and RDA2 explained 23.34%. Similarly, SWC, SOC, AP, AN, TK, TN, and TP were the primary influencing factors on fungal community structure. The influence of pH on both bacterial and fungal structures is inversely proportional; higher structural diversity indicates lower pH values (Figure 9b).

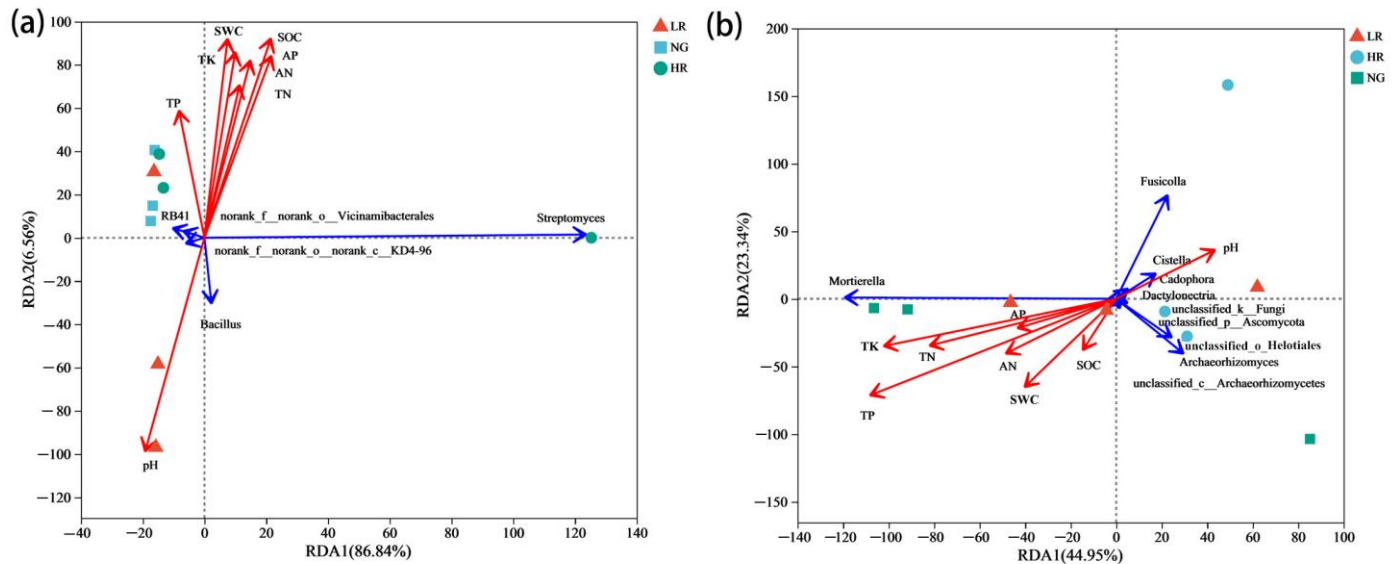


Figure 9. Redundancy analysis (RDA) of bacterial communities (a) and fungal communities (b) with soil properties in alpine mining grassland with various restoration levels. Soil physicochemical properties indicated in red, bacterial dominant species in blue (a), fungal dominant species in blue (b). LR, low restoration; HR, high restoration; NG, natural grassland. SWC, soil water content; SOC, soil organic carbon; EC, electrical conductivity; AN, available nitrogen; AP, available phosphorus; TK, total potassium; TN, total nitrogen; TP, total phosphorus.

4. Discussion

4.1. Effects on Vegetation and Soil Physicochemical Properties in High-Altitude Mining Area Grassland Restoration

The development of grassland ecosystems is closely tied to vegetation and soil dynamics. Vegetation characteristics directly reflect soil properties, and soil features are fundamental criteria for assessing vegetation growth [41]. In our study, HR mining area grasslands exhibited a higher density, height, and Shannon–Wiener index compared to LR grasslands (Table 1). This positive correlation with restoration level and vegetation features indicates that improved vegetation growth reflects a higher level of restoration, providing a visual indicator of grassland recovery. As the restoration level of the mining area grasslands increased, soil organic carbon, total phosphorus, total nitrogen, available phosphorus, nitrate nitrogen, ammonium nitrogen, and alkaline nitrogen content showed an increasing trend. This is primarily attributed to the gradual accumulation of nutrients in the soil with the increasing restoration level. Despite lower precipitation in high-altitude grasslands, the higher water content in the surface soil, coupled with the ability of plants and root systems to inhibit surface runoff infiltration, results in enriched microbial populations and root exudates in the surface soil [42]. Consequently, as the restoration level increases, soil microbial activity significantly rises, leading to a noticeable increase in soil nutrient levels.

In this study, higher levels of soil water content (SWC), soil organic carbon (SOC), available nitrogen (TN), total phosphorus (TP), total potassium (TK), available phosphorus (AP), and available nitrogen (AN) in the HR mining area were likely attributed to increased soil mineralization, animal feces, plant secretions, and litter accumulation with the progression of restoration, lowest content levels of soil pH and electrical conductivity (EC) (Figure 2). Elevated restoration levels are associated with a decline in pH and EC. This is likely attributed to heightened organic carbon production and decomposition, leading

to decreased pH, and the removal of salts, resulting in lower EC [30]. Grassland recovery may exhibit synchronous degradation or improvement in both aboveground vegetation and soil; soil recovery exhibits a lag compared to the recovery of aboveground vegetation communities. The nutrient content in the soil surface layer was more influenced, consistent with the observed pattern of soil nutrient changes in this study. TP and TN in the soil, representing the sum of various forms of phosphorus and nitrogen, were significantly positively correlated with soil organic matter. In LR sites, where aboveground vegetation was sparse, and surface coverage was low, prolonged wind erosion led to lower levels of SWC, SOC, TN, TP, TK, AP, and AN. The mining area's grassland restoration in HR sites showed optimal results, reaching vegetation and soil chemical characteristic levels similar to those of natural grasslands after a certain period. Grassland degradation and restoration constitute a slow process. Human-assisted restoration can expedite vegetation growth and soil property enhancement, but adaptation to the harsh conditions of high-altitude grasslands and the formation of a stable grassland ecosystem require time [43]. This aligns with the findings of Gao et al. [44], emphasizing that soil recovery is a gradual process leading to an increase in land's potential utility value and productivity, marking a slow but steady restoration of high-altitude mining area grassland ecosystems.

4.2. Effects of Grassland Restoration on Soil Microbial Communities in Alpine Grassland Mining Areas

Soil microbial communities are highly sensitive to environmental changes. In our study, the restoration of alpine grasslands in high-altitude mining areas significantly influenced the dynamics of soil microbial communities, including bacteria and fungi. At different restoration levels, the dominant bacterial phyla were Actinobacteriota, Proteobacteria, Myxococcota, Acidobacteriota, and Verrucomicrobiota. The dominant fungal phyla were Ascomycota, Olpidiomyota, Mortierellomycota, Glomeromycota, and Basidiomycota. Notably, the relative abundance of these phyla differed significantly between the LR and HR levels, and their abundance also varied between HR and the undisturbed NG reference (Figure 4). These differences can be attributed to shifts in plant community composition due to grassland restoration [45]. Restoration areas may also experience variations in vegetation cover due to factors such as grazing disturbances, differences in elevation, or proximity to roadways, resulting in variations in soil nutrient content and the composition and structure of soil bacterial and fungal microbial communities [46,47].

Among the different levels of restoration in high-altitude mining area grasslands, HR had the highest number of shared operational taxonomic units (OTUs) with NG (Figure 2). This indicates that the highest level of restoration, HR, closely resembled NG in terms of soil microbial community composition [48]. In LR restoration areas, the relative abundance of copiotrophic bacteria (Proteobacteria and Firmicutes) was the highest, while oligotrophic bacteria (Acidobacteria and Chloroflexi) had the lowest relative abundance. In HR restoration areas, Acidobacteria had the highest relative abundance, and Proteobacteria had a relative abundance similar to HR and NG (Figure 7). This suggests that HR, with the highest vegetation cover, achieved better restoration results and approached the microbial community composition of NG over time with regular management [49]. In LR areas with lower vegetation cover, the relatively high abundance of copiotrophic bacteria (Proteobacteria and Firmicutes) can be attributed to the lower levels of nutrients in the LR mining area grasslands. Copiotrophic bacteria tend to thrive in environments where resources are limited, leading to an R-strategy proliferation in response to nutrient competition [50]. As a result, LR areas with the lowest vegetation cover, and therefore poorer restoration results, exhibited the highest relative abundance of copiotrophic bacteria compared to HR and NG areas. The extensive proliferation of copiotrophic bacteria, as they compete for limited resources, restricts the growth of oligotrophic bacteria, leading to their lowest relative abundance [51].

Soil fungal communities exhibit variations across different restoration levels, primarily dominated by the phyla Ascomycota and Basidiomycota [52]. In low-restoration

(LR) grasslands, the relative abundance of Basidiomycota significantly decreases, while in high-restoration (HR) grasslands, Ascomycota dominates, accompanied by the highest relative abundance of Mortierellomycota at the genus level. The restoration level of HR grasslands closely resembles that of natural grasslands (NG). Some studies suggest that grassland restoration impacts soil organic matter content, with higher organic matter associated with diverse bacterial communities and greater fungal diversity in low-organic matter environments [53]. Soil fungal communities exhibit differential sensitivity to environmental disturbance and soil moisture, with higher moisture levels suppressing fungal growth. Additionally, research indicates that Ascomycota thrives in environments with a higher soil lignin content, primarily decomposing woody vegetation debris. In high-altitude grasslands, soil lignin content is typically elevated, aligning with the findings of this study [54,55].

4.3. Impact of Vegetation and Soil Environmental Factors on Soil Microbial Communities in Alpine Grassland Mining Areas

During the restoration process of high-altitude mining areas grasslands, vegetation and soil environmental factors have distinct impacts on soil microbial communities. Vegetation cover, soil moisture, organic carbon, total nitrogen, total phosphorus, and total potassium are closely related to soil fertility, which directly influences plant growth and microbial community activity [56]. In high-altitude mining area grasslands, the soil is affected by long-term exposure to high altitudes and low temperatures, leading to poor soil structure and permeability, which to some extent inhibits the proliferation of soil microbes [57]. The redundancy analysis (RDA) in this study (Figure 9) reveals that soil bacterial and fungal communities are primarily regulated by changes in soil factors such as soil water content (SWC), soil organic carbon (SOC), available nitrogen (TN), total phosphorus (TP), total potassium (TK), available phosphorus (AP), and available nitrogen (AN). HR grasslands are found to be closer to NG natural grasslands in terms of their soil properties, indicating more effective restoration. In NG mining area grasslands, the highest soil fertility and nutrient levels lead to optimal plant growth. However, HR grasslands, with the highest vegetation cover, demonstrate the same degree as NG, showcasing successful restoration efforts. Regular monitoring and maintenance will inevitably result in the rapid recovery of these areas to a healthy pre-mining state over time.

The changes in soil microbial communities are primarily driven by the influences of vegetation and soil environmental factors [58]. The relationships among vegetation, soil, and microbes are interconnected and mutually influential [59]. In HR mining area grasslands, the soil bacterial microbial community showed the highest richness indices; particularly, the Chao1 and ACE indices significantly increased. Conversely, in NG mining area grasslands, the soil fungal microbial community exhibited the highest richness indices, including the Simpson and Chao1 indices, which also significantly increased (Figure 5). This might be attributed to the relatively higher soil fertility in HR and NG grasslands, as soil fertility directly impacts the abundance of soil microbial communities, consistent with findings in other studies [60]. The varying degree of mining-induced damage, differences in vegetation cover during later restoration stages, and the specific characteristics of mining area grasslands, which often feature sloping terrain and variation in the presence of large shrubs or bushes, can lead to differing restoration outcomes. The presence of large shrubs, for instance, may affect light exposure and, subsequently, the success of restoration efforts [61]. Grazing and human trampling can also have a significant impact on post-restoration outcomes. Since restoration areas are typically large and interconnected with their natural grassland counterparts, they cannot be entirely fenced off; as a result, external disturbances, such as grazing by livestock, can lead to varying degrees of restoration success [62]. In summary, LR and HR mining area grasslands experience significant changes in soil microbial communities during the restoration process. In high-altitude mining area grasslands, the influence of soil characteristics is more direct than the influence of vegetation characteristics. However, the trends in above-ground vegetation largely

determine the overall trends in soil properties, aligning with the findings of many other research studies [63,64].

5. Conclusions

This study has demonstrated that increasing the restoration level of high-altitude mining area grasslands significantly enhances the content of soil physicochemical properties, including SWC, SOC, TN, TP, TK, AN, and AP, while decreasing soil pH and EC levels. Additionally, restoration grassland in alpine mining areas significantly altered the distribution and composition of bacterial and fungal communities in soils but had no significant effect on the diversity of microbial communities. Notably, as the restoration degree of high-altitude mining area grasslands increases, the dominant species in the bacterial community transition from copiotrophic bacteria (Proteobacteria and Firmicutes) to oligotrophic bacteria (Acidobacteria and Chloroflexi). In the case of fungal communities, the distribution and composition were most abundant in HR mining area grasslands, and the restoration level in HR was close to that of NG. Furthermore, the restoration process of high-altitude mining area grasslands was influenced by changes in plant growth and alterations in soil environmental factors, both of which contribute to shifts in soil microbial community composition. Importantly, the impact of changes in soil properties and environmental factors outweighs the influence of plant growth on soil microbial community composition. The changes in soil microbial community composition were primarily regulated by alterations in soil properties, such as SOC, SWC, TP, AP, AN, TN, and TK. The HR mining area grasslands closely resemble the natural grassland NG, indicating a more successful restoration effect. Our research findings enhance our understanding of changes in plant, soil, and microbial community composition. They illustrate that the restoration of high-altitude mining area grasslands facilitates transformations in plant, soil, and microbial community dynamics. This study provides a scientific foundation and data to support ongoing restoration and management efforts in the Qilian Mountains alpine mining grassland. It also contributes to the theoretical framework for high-altitude mining area grassland restoration, offering valuable insights for future restoration initiatives.

Author Contributions: X.Y. and Q.F.: conception and design of the research. X.Y., M.Z. and L.Y.: acquisition of data and drafting the manuscript. X.Y. and C.Z.: analysis and interpretation of data. X.Y. and J.Z.: statistical analysis. Y.F., Z.W. and L.Y.: revision of manuscript for important intellectual content. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key R&D Program of China (No. 2022YFF1303301, 2022YFF1302603), the National Natural Science Fund of China (No. 52179026, 42001035, 42101115), the Science and Technology Program of Gansu Province (No. 22JR5RA072, 22JR5RA068), the Post-doctoral Funding Program of Gansu Province (No. E339880139), the Natural Science Foundation of Gansu Province (No. E331040901), the Science and Technology Fund of Gansu Province (No. 23JRRA640), and the Open Fund of Technology Innovation Center for Mine Geological Environment Restoration in the Alpine and Arid Regions (No. HHGCKK2204).

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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