

## Article

# Effects of Vegetation Restoration on Soil Bacterial Communities, Enzyme Activities, and Nutrients of Reconstructed Soil in a Mining Area on the Loess Plateau, China

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**Abstract:** Soil microbes are the main driving forces and influencing factors of biochemical reactions in the environment. Study of ecological recovery after mining activities has prompted wider recognition of the importance of microbial diversity to ecosystem recovery; however, the response of soil bacterial communities to vegetation restoration types and soil biochemical properties remains poorly understood. The purpose of this research was to explore the soil bacterial communities and soil biochemical properties at four sampling sites (brushland (BL), forestland (FL), grassland (GL) and unreclaimed land (UL)) on the Loess Plateau, China, to evaluate the effect of vegetation restoration on the reconstructed soil in mining areas. In August 2017, samples were collected at the Heidaigou coal mine dumps. Illumina MiSeq sequencing was used to identify the structure of the soil bacterial community and evaluate its relationships with soil biochemical properties. The results showed that soil biochemical properties (soil organic matter, available phosphorus, urease, sucrase, microbial biomass carbon and microbial biomass nitrogen) were significantly increased in BL, FL and GL relative to UL, indicating that the soil quality was significantly improved by vegetation restoration. In addition, the results showed that the vegetation restoration on the reconstructed soil in the mining area could significantly improve the operational taxonomic units (OTUs), abundance (ACE and Chao1) and diversity (Shannon and Simpson) indices of bacterial community and the dominant phyla were Proteobacteria, Actinobacteria and Acidobacteria. With vegetation restoration, the relative abundance of Proteobacteria and Acidobacteria showed an increasing trend, while that of Actinobacteria showed a decreasing trend, and the dominant phyla were only significantly correlated with a few biochemical properties. Moreover, there were no changes in soil bacterial community structures across the four sampling sites and the response of the bacterial community to biochemical properties was not obvious. This implies that, although the region has experienced about 20 years of vegetation restoration, the microbial community still maintains good stability and lagging response to soil biochemical properties. Since the BL soil had better biochemical properties and higher bacterial richness and diversity, it was recommended as the optimum vegetation restoration type for soil reclamation in this area.

**Keywords:** high-throughput sequencing; vegetation restoration; reconstruction soil; enzyme activities; the Loess Plateau

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## 1. Introduction

Mining activities causes extensive damage to soil and changes the soil environment, adversely altering soil properties and the function and stability of microbial community structure [1]. The Loess Plateau region of China is one of the most serious areas of soil erosion and one of the most vulnerable ecological environments in the world [2]. The final objective of ecological restoration and reclamation in mining areas is to restore productivity on the post-mining land and maintain the sustainable development of ecosystem [3,4].

Microbes represent the largest part of the Earth's biodiversity and bacteria are the most abundant and diverse of the soil microbes [5]. Soil microbes play a crucial role in ecosystem processes and are the main driving forces and influencing factors of biochemical processes [6]. Due to the important mediating role of soil microbes in ecosystems, the restoration of the soil microbial community is a key process of soil restoration and plays a positive role in achieving soil health and sustainable utilization [4]. Studies have shown that soil microbes play a major role in regulating a variety of ecological functions [7,8]. Microbes obtain nutrients and habitats from the soil and have an impact on soil quality [9].

In view of the integrated role of microbes in energy migration, nutrient cycling and vegetation restoration, exploration of the relationship between different vegetation restoration conditions and soil microbial communities can provide important information for ecological restoration. Chen et al. [10] reported that removal of vegetation reduced soil substrate and microbial food sources, thus leading to a reduction in biomass or abundance of microbial. Enzymes produced by plants and microbes are important in nutrient cycling and flows of energy in soil. For example, sucrase (SUC), urease (URE) and alkaline phosphatase (ALP) are closely related to the C, N and P cycles of soil [11]. Enzymes play an important role in many soil ecological processes and are closely related to the proliferation of soil microbial communities [12]. Moreover, the enzyme activity can rapidly respond to the changes of microbial community under vegetation restoration.

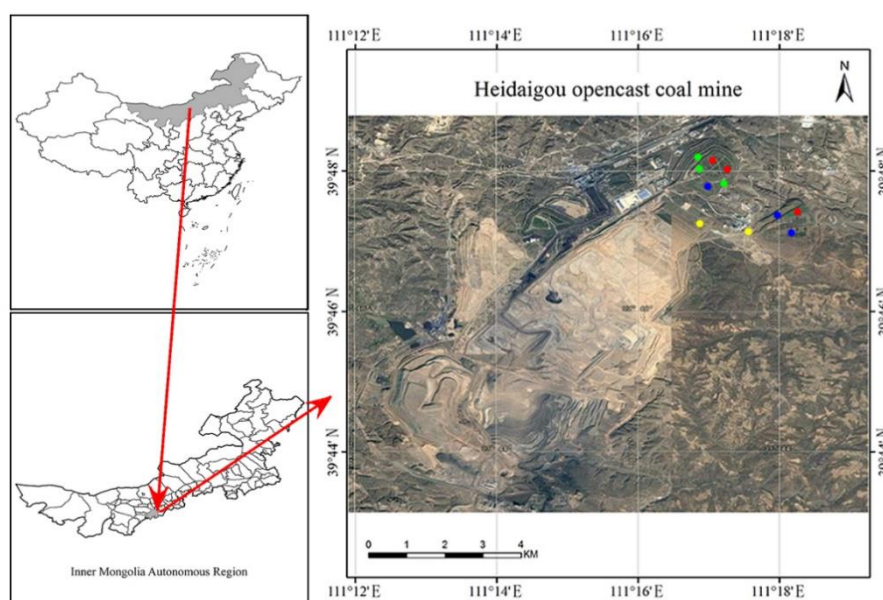
Previous studies have assessed the reclamation effectiveness of the reconstructed soil in mining areas, focusing on soil quality [13], physicochemical characteristics [14–16] and vegetation characteristics [17]. Although microbes play a vital role in ecosystem processes and even can serve as an indicator to evaluate the recovery status of the damaged environment [18], there remains insufficient information about the relationship among soil properties, soil microbial communities and vegetation. Such information is necessary for us to better comprehend and evaluate the ecological restoration effect of mine reclamation.

In recent years, high-throughput sequencing technology has greatly promoted the development of microbial community research and provided us with new tools to understand microbes in the environment [19]. In this study, we conducted high-throughput sequencing of the bacterial 16S rRNA gene at four sampling sites with different vegetation types on reconstructed soil in the Loess Plateau mining refuse dumps for quantitative research on the soil bacterial communities. In addition, biochemical properties including enzyme activities, nutrients and microbial biomass of soil were measured. The objectives of the study were: (1) to evaluate the changes of soil biochemical properties during artificial vegetation restoration on reclaimed land; (2) to determine the diversity and structure of soil bacterial community under different vegetation restoration; and (3) to identify the relationship between soil bacterial community structure and biochemical properties during vegetation restoration.

## 2. Materials and Methods

### 2.1. Study Area and Soil Sampling

The research was carried out in the Heidaigou opencast mine located in the southeast of Zhungeer Banner, Inner Mongolia Autonomous Region, China ( $39^{\circ}43'–39^{\circ}49'$  N and  $111^{\circ}13'–111^{\circ}20'$  E; altitude of 1025–1302 m) (Figure 1). The landform of the mining area is a typical loess hilly gully region, with a semi-arid, temperate continental climate. The average annual precipitation is 401.6 mm, which is concentrated from July to September, accounting for about 60–70% of annual precipitation [20] and the annual evaporation ranges from 1824.7–2896.1 mm. The zonal soil is castano-cinnamon soil with sandstone as the mother rock and the soil of the mining area is characterized by loessial soil, with loose soil, poor erosion resistance, slight alkalinity and low fertility [21]. The severe disturbance of mining has caused great changes in the inherent physicochemical and biological characteristics of the local soil, mainly in the form of serious damage to the soil aggregates, serious loss of nutrients and topographic changes [20]. Generally, the soil of dumps is mixed with particles of various sizes and rock fragments [17], which can be considered a type of reconstructed soil.



**Figure 1.** Geographical location of the study region and sampling sites. Blue dots represent the brushland, red dots represent the forestland, green dots represent the grassland and yellow dots represent the unreclaimed land.

There are five refuse dumps in the Heidaigou opencast coal mine, at which vegetation construction started in 1995, 1998, 2003, 2005 and 2014, respectively. We chose two of the largest refuse dumps for the study area: the northern dump, which began vegetation construction in 1995 and the eastern dump, which began vegetation construction in 1998 [17]. Although vegetation restoration succession was a slow process, the reclamation of dumps by revegetation can improve soil quality and establish a relatively stable ecosystem. The main plant species in the study area are *Pinus tabuliformis*, *Populus beijingensis*, *Caragana microphylla*, *Hippophae rhamnoides*, *Artemisia desertorum*, *Medicago sativa* and *Agropyron cristatum*. To minimize the impact of soil characteristics on soil properties, all the sampling sites had similar topography, altitude, similar geographical coordinates and little difference in recovery time, which ranged between 18 and 20 years in this study. The basic information on the sampling sites is shown in Supplementary Materials Table S1.

In August 2017, three plots were established on brushland (BL), forestland (FL) and grassland (GL). Since the area of unreclaimed land was small, only two plots were established on unreclaimed

land (UL). Five soil cores (5 cm diameter, 0–20 cm) were randomly collected using a soil auger from each individual plot and then mixed evenly to create a compound sample. Each mixed sample was immediately passed through a 2-mm sieve and then compartmentalized into three portions for further analysis. The first portion was kept at  $-80^{\circ}\text{C}$  for subsequent DNA extraction. The second portion was kept at  $4^{\circ}\text{C}$  for enzyme activity, microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) analysis. The third portion was retained for analysis of chemical properties after experimental pretreatment.

## 2.2. Analysis of Soil Chemical and Biological Properties

Soil moisture was determined gravimetrically in fresh soils at  $105^{\circ}\text{C}$  overnight. Soil pH was measured in soil-water solution (1:5) by pH meter (PHSJ-3F pH Meter, INESA Scientific Instrument Co., Ltd., Shanghai, China) and soil organic matter (SOM) and available nitrogen (AN) were measured using the modified Walkley–Black method [22] and alkaline hydrolysis diffusion [23], respectively. Available phosphorus (AP) was extracted with the sodium bicarbonate and then determined by colorimetry [24]. We used potassium permanganate titration, disodium phenyl phosphate colorimetry, indophenol-blue colorimetry and 3,5-dinitrosalicylic acid colorimetry to measure catalase (CAT), ALP, URE and SUC activities, respectively [25,26]. The MBC and MBN were analyzed by the chloroform fumigation-extraction method [27]. Three replicated tests were performed for all the above indicators.

## 2.3. DNA Extraction, High-throughput Sequencing and Data Analysis

Total bacterial DNA were extracted from samples employing the Power Soil DNA Isolation Kit (MO BIO Laboratories) according to the manufacturer's instruction. The DNA quantity and quality were evaluated by microspectrophotometry (Eppendorf, Germany) and 1% agarose gel electrophoresis [4]. Then DNA was kept at  $-80^{\circ}\text{C}$  until further processing. The V3–V4 region of the bacterial 16S rRNA gene was amplified with the primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and primer 806R (5'-GACTACHVGGGTWTCTAAT-3') [28]. PCR amplification system (50  $\mu\text{L}$ ): 338F/806R (10  $\mu\text{mol}$ ) 2  $\mu\text{L}$  of each respectively, 50 ng genome DNA (1  $\mu\text{L}$ ), d NTPs (10  $\text{mmol L}^{-1}$ ) 1  $\mu\text{L}$ , High GC Enhancer 10  $\mu\text{L}$ , Q5 High-Fidelity DNA Polymerase 0.2  $\mu\text{L}$ , Buffer 10  $\mu\text{L}$ . The polymerase chain reaction (PCR) procedure was as follows:  $95^{\circ}\text{C}$  pre-denaturation for 3 min,  $95^{\circ}\text{C}$  modified for 30 s and  $50^{\circ}\text{C}$  annealing for 30 s,  $72^{\circ}\text{C}$  extension for 60 s, 30 cycles; and finally,  $72^{\circ}\text{C}$  extension for 7 min. Three duplicate PCR products were tested with 2% agarose gel electrophoresis and pooled, then quantified using the AxyPrepDNA gel extraction kit (AXYGEN Corporation, USA) and the QuantiFluor™-ST blue fluorescence quantitative system (Promega Corporation, USA). Finally, a total of 637,635 sequenced reads from all the purified and pooled samples were analyzed by high-throughput sequencing using the Illumina Hiseq 2500 platform (Illumina Corporation, USA) at Biomarker Technologies Corporation, Beijing, China.

The Trimmomatic software platform (Version 0.33) was used for de-noising, sorting and distinguishing the original sequence and then primers were trimmed [29]. Redundancy screening was performed for the remaining sequences and all of the unique sequences for each sample were clustered as operational taxonomic units (OTUs) with 97% similarity using Mothur (<https://www.mothur.org/wiki/Chao>) [30] on July 5, 2018. The bacterial 16S rRNA Silva reference database (<http://www.arb-silva.de>) was used to classify and identify the representative sequences of each OTU and ribosomal database project (RDP) naïve Bayesian classifier at confidence level of 80% to assign the taxonomic composition [31].

Community richness index (Chao1 and ACE estimator), community diversity index (Shannon and Simpson index) and rarefaction curves were calculated in Mothur using the high-throughput sequencing data [9]. The 16S rRNA high-throughput sequencing data were submitted to the NCBI Sequence Read Archive (SRA) with the Submission ID of SUB5220565.

## 2.4. Statistical Analysis

The data analyses were performed in SPSS 22.0 for Windows (SPSS Inc., Chicago, USA). Pearson's correlation analysis was performed to determine the relationships among the soil properties and relative abundance of soil bacteria. One-way analysis of variance (ANOVA) was employed to examine the significant differences under the different sampling sites (soil chemical properties, biological properties and bacterial community richness and diversity indices). Significant differences were determined by the least significant difference (LSD) test at 0.05 probability. The histogram was drawn using Microsoft Excel (2016). A Venn diagram was established based on 97% similarity of OTUs per sample to visually show the overlaps and differences among different sampling sites using the VennDiagram package in R [32]. A heatmap was drawn using R (<http://127.0.0.1:22929/library/stats/html/heatmap.html>) from the most abundant OTUs in the bacterial community. The hierarchical cluster dendrograms based on weighted UniFrac were used to visually show the similarity and dissimilarity of bacterial community structures across all of the soil sampling sites. According to the UniFrac distance between different samples, principal coordinate analysis (PCoA) was used to assess the overall variations of microbial community structure. Adonis analysis was carried out on the sampling sites under different vegetation restoration types to detect whether there were significant differences between bacterial communities. After Hellinger transformation of bacterial species data and standardization of environmental data, redundancy analysis (RDA) was used to confirm the influence of environmental factors on the bacterial community in R using the vegan package.

## 3. Results

### 3.1. Soil Biochemical Properties

The effect of vegetation restoration on soil biochemical properties is presented in Figure 2. Soil biochemical properties displayed significant differences across the four sites ( $P < 0.05$ ) (except pH and ALP). The soil moisture of the three vegetation restoration types was significantly higher than that of the unreclaimed land ( $P < 0.05$ ). The BL had the highest values of SOM ( $23.41 \text{ g} \cdot \text{kg}^{-1}$ ), URE ( $1.63 \text{ mg NH}_4^+ \cdot \text{N g}^{-1} \cdot 24\text{h}^{-1}$ ), MBC ( $475.17 \text{ mg} \cdot \text{kg}^{-1}$ ) and MBN ( $92.74 \text{ mg} \cdot \text{kg}^{-1}$ ). The lowest contents of SOM ( $8.73 \text{ g} \cdot \text{kg}^{-1}$ ), URE ( $0.53 \text{ mg NH}_4^+ \cdot \text{N g}^{-1} \cdot 24\text{h}^{-1}$ ), SUC ( $3.32 \text{ mg glucose g}^{-1} \cdot 24\text{h}^{-1}$ ), MBC ( $156.44 \text{ mg} \cdot \text{kg}^{-1}$ ) and MBN ( $17.78 \text{ mg} \cdot \text{kg}^{-1}$ ) were observed in the UL, whereas the AP ( $12.25 \text{ mg} \cdot \text{kg}^{-1}$ ) in the GL was higher than those in the other three sites.

### 3.2. Compositions and Structures of Bacterial Communities

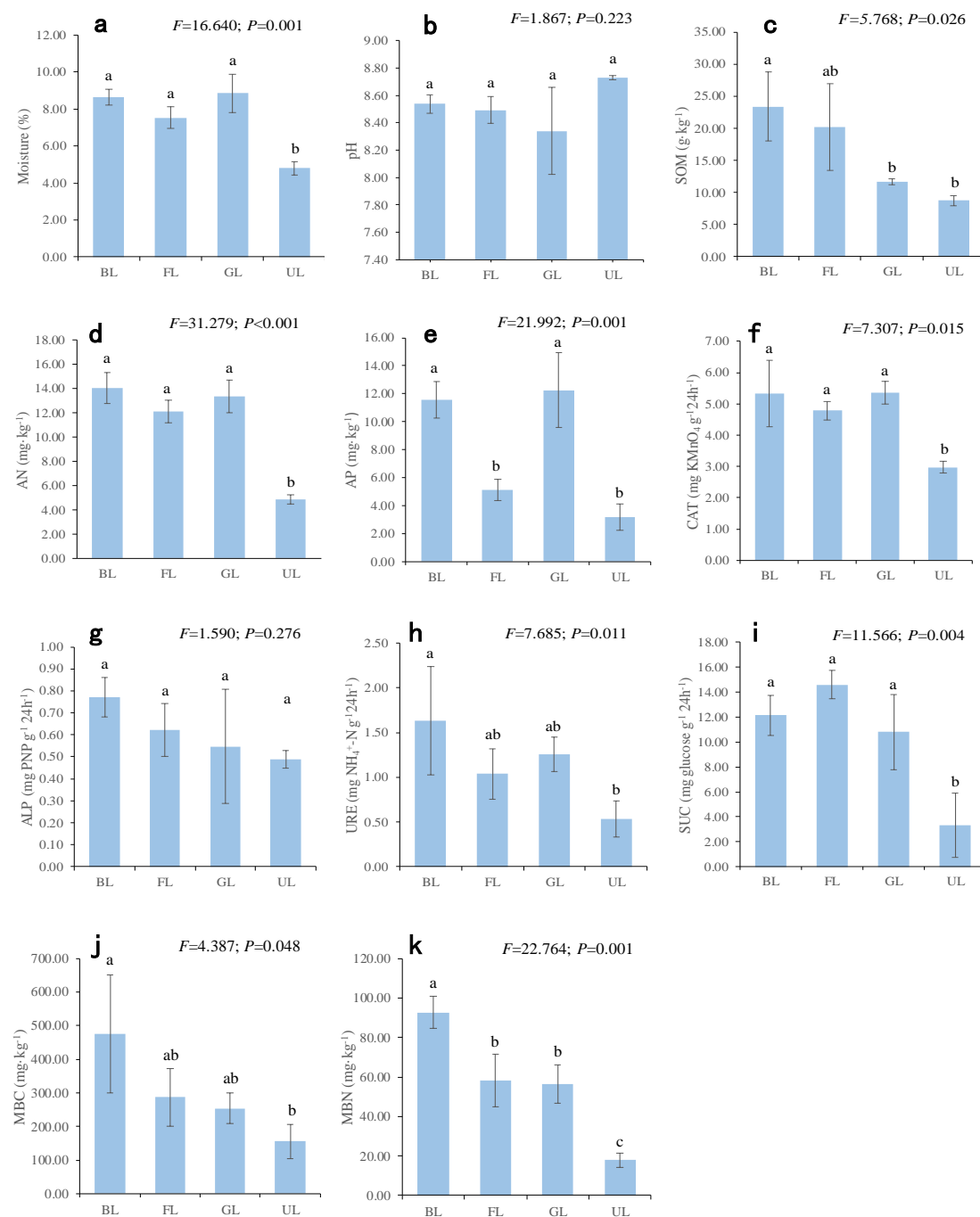
After the quality trimming and removal of chimeras, 459715 high-quality sequences were retained from the integrated data set (an average of 41792 per sample for bacterial communities). A total of 1927 OTUs were detected according to 97% similarities. At the phylum level, the overall bacterial community compositions of the samples were similar, whereas different proportions of some samples were observed (Figure 3). Proteobacteria was the most abundant phylum in all of the samples (40.82% on average), ranging from 37.27% to 46.30%. Actinobacteria was the second most abundant phylum, with an average relative abundance of 22.14%. Other dominant phyla in decreasing order of the average value were: Acidobacteria (11.28%), Gemmatimonadetes (8.81%), Bacteroidetes (7.66%), Chloroflexi (5.05%), Nitrospirae (0.77%), Verrucomicrobia (0.68%), Saccharibacteria (0.58%) and Cyanobacteria (0.51%).

At 97% similarity level, rarefaction curves of all of the samples gradually leveled off, demonstrating that the amount of sequence data was adequate and the continuous increase in the number of reads made almost no contribution to the total number of OTUs (Supplementary Materials Figure S1).

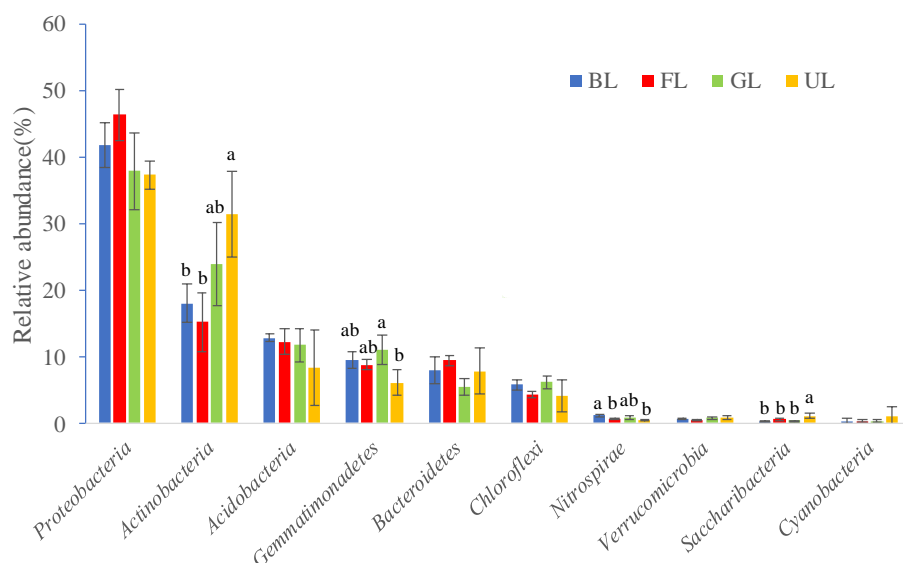
Statistically significant differences in richness (ACE and Chao1) and diversity (Shannon and Simpson indices) were observed under different sampling sites (Table 1;  $P < 0.05$ ). The coverage of all samples was above 98%, indicating that the results of sequencing were reliable and reflected the real situation of soil bacteria. There was no significant difference in the ACE, Chao1 estimators and Shannon index between the BL, FL and GL (Table 1); however, the lowest ACE, Chao1 estimators and



Shannon index were observed in UL. These findings indicate that vegetation restoration significantly improved the bacterial community richness and diversity compared with the UL.



**Figure 2.** Soil biochemical properties (a–k indicate moisture, pH, SOM, AN, AP, CAT, ALP, URE, SUC, MBC and MBN) of the different sites. Different lowercase letters indicate significant differences ( $P < 0.05$ ) among the different sites under the same indicator based on one-way ANOVA followed by LSD test. The  $F$  and  $P$  values were the results of the ANOVA. BL, brushland; FL, forestland; GL, grassland; UL, unreclaimed land.



**Figure 3.** Relative abundance of soil bacterial communities at the phylum level (ranks top 10). Relative abundance is the ratio of the abundance of a sequence type to the total number of sequences. Data were analyzed by one-way analysis of variance and means were compared by LSD test. Different letters indicate significant differences ( $P < 0.05$ ) among the different sampling sites.

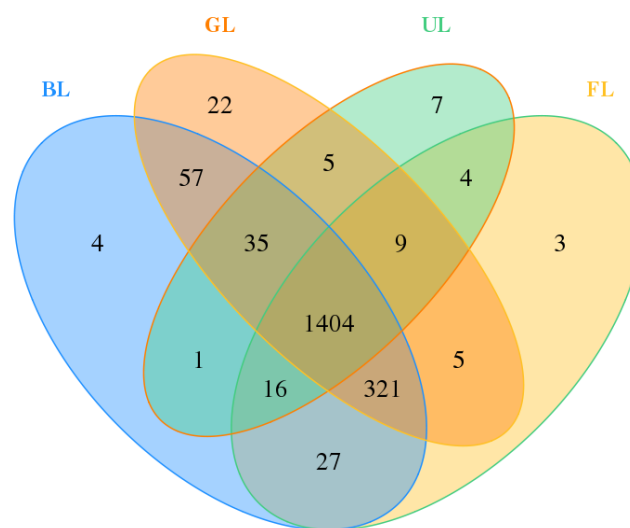
**Table 1.** Bacterial community richness and diversity indices of the different sites.

Site	OTUs	ACE	Chao1	Shannon	Simpson	Coverage
BL	1664 ± 56a	1749 ± 46a	1773 ± 35a	6.26 ± 0.07a	0.006 ± 0b	0.994 ± 0a
FL	1579 ± 50a	1668 ± 30a	1675 ± 32a	6.03 ± 0.04a	0.009 ± 0ab	0.994 ± 0a
GL	1492 ± 10a	1593 ± 34a	1624 ± 44a	6.21 ± 0.05a	0.006 ± 0b	0.993 ± 0a
UL	1118 ± 264b	1331 ± 192b	1335 ± 193b	5.59 ± 0.50b	0.012 ± 0a	0.986 ± 0a
<i>F</i>	11.288	11.628	12.354	5.479	4.477	2.792
<i>P</i>	0.005	0.004	0.003	0.030	0.047	0.119

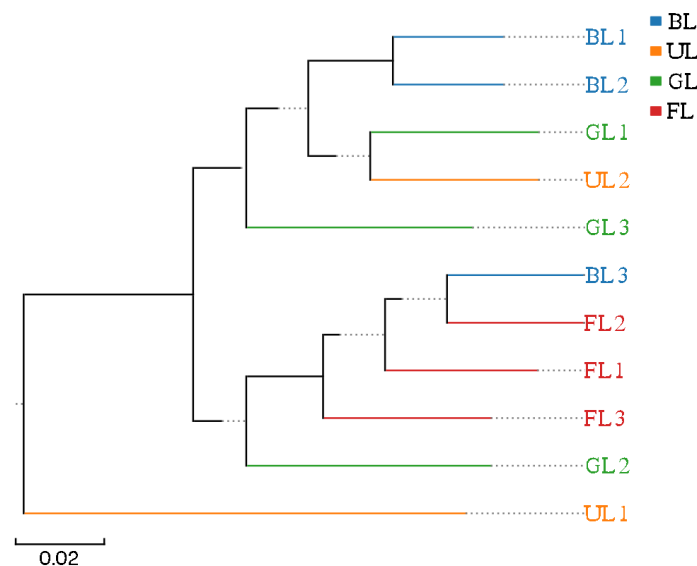
Note: Different lowercase letters indicate significant differences ( $P < 0.05$ ) among the different sites under the same indicator based on one-way ANOVA followed by LSD test. OTUs, operational taxonomic units; ACE, abundance-based coverage estimator; Coverage, Good's nonparametric coverage estimator; Shannon, nonparametric Shannon diversity index; Simpson, nonparametric Simpson diversity index.

The beta diversity of soil bacterial community data was analyzed to test whether these community patterns were different under vegetation restoration types. The Venn diagram generated based on OTUs displayed that a total of 1404 OTUs were shared by all sites (Figure 4).

To compare the similarity and difference among all of the sample sites, hierarchical cluster analysis and PCoA based on weighted UniFrac distances were conducted (Figures 5 and 6). It was found that the composition of bacterial communities was consistent under different vegetation restoration types. This was also confirmed by Adonis analysis (Supplementary Materials Table S2). Results of the hierarchical clustering showed three main categories and the majority of bacterial communities procured from BL were similar to GL; however, the homogeneity of bacterial community in the UL plots was poor. Furthermore, PCoA clearly showed the changes of bacterial community among soil samples under different vegetation restoration types (Figure 6). The first and second axis explained 49.83% and 30.42% of the variance, respectively. The BL, GL and FL sites were separated from the UL site along second axis and the UL was separated from the other sites along the first axis. In addition, PCoA based on weighted UniFrac distances showed that the bacterial communities of the UL were obviously different from those of the BL, GL and FL samples and a few sites overlapped in the BL and GL groups.



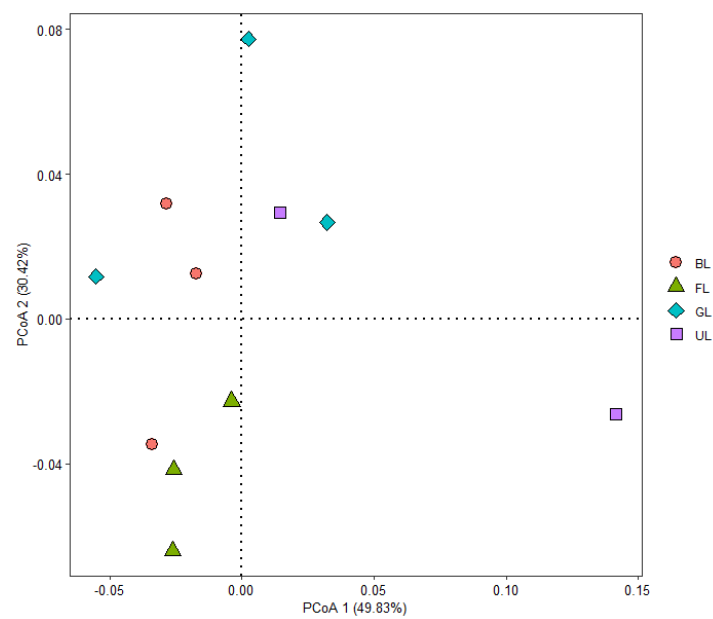
**Figure 4.** Venn diagram showing unique and shared operational taxonomic units (OTUs).



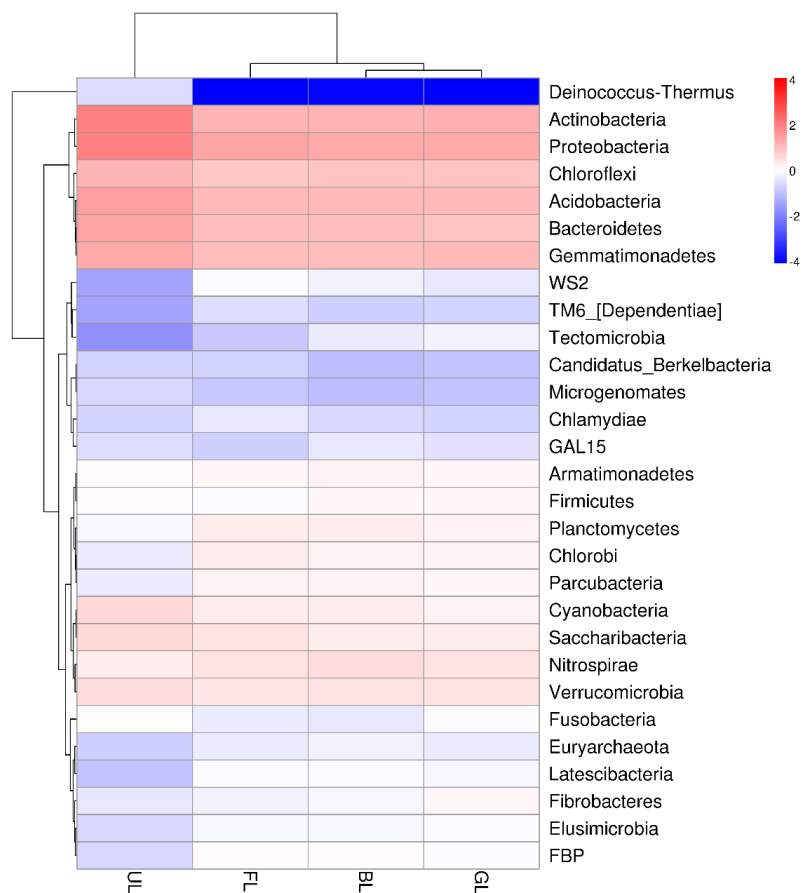
**Figure 5.** Hierarchical cluster dendrogram of soil bacterial communities based on the weighted UniFrac distance of the different sampling sites.

As shown in Figure 7, the bacterial community abundance of the four sampling sites was separated into two groups at the first level. The first comprised the UL, whereas the other comprised the FL, BL and GL, demonstrating that communities in the FL, BL and GL were similar and formed a branch outside of the UL. The heatmap showed that Actinobacteria, Proteobacteria and Acidobacteria were the predominant phyla in all of the sites (Figure 7). There were some differences in relative abundance between the UL and vegetated restoration land (GL, BL and FL). Figure 7 illustrates that the relative abundance of the GL was more similar to that of the BL sites and both of sites also showed a clear difference from the UL. Compared with UL, vegetated restoration results indicated significant variations in relative abundance of bacterial community to some extent, especially in Actinobacteria, Gemmatimonadetes, Nitrospirae and Saccharibacteria (Figures 3 and 7).





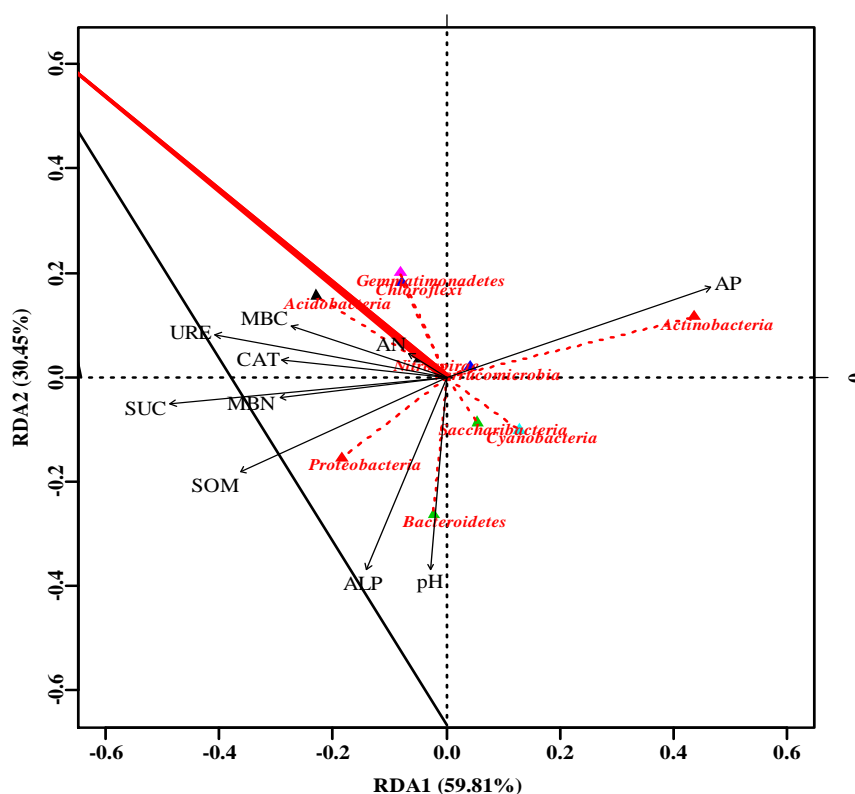
**Figure 6.** Principal coordinate analysis (PCoA) of soil bacterial communities from different sampling sites.



**Figure 7.** Heatmap of abundance in the bacterial community of different sampling sites. The values after logarithmic transformation are indicated by color intensity with the legend indicated in the top right corner.

### 3.3. Relationships between Bacterial Communities and Soil Biochemical Properties

The effects of soil biochemical properties on bacterial communities were investigated by RDA (Figure 8). The first two axes account for 90.26% of the total variation in the bacterial community structures. The RDA diagram clearly shows that SUC, SOM and MBN were concentrated in the same direction, whereas MBC, URE, CAT and AN were concentrated in the other direction. Moreover, AP, SUC, ALP and URE have longer arrows, indicating that they were primary properties influencing bacterial communities. We found that major bacterial phyla, such as Proteobacteria, Acidobacteria and Gemmatimonadetes, were spread in Quadrants II and III, except for Actinobacteria which spread in Quadrant I. In addition, we also calculated the Pearson's correlation coefficient (Supplementary Materials Table S3) between the top 10 bacterial communities at the phylum level and the soil biochemical properties. The relative abundance of Acidobacteria was positively correlated with AN ( $P < 0.01$ ) and CAT ( $P < 0.05$ ) and Actinobacteria was negatively correlated with AN ( $P < 0.05$ ) and MBN ( $P < 0.05$ ). The relative abundance of Proteobacteria was positively correlated with ALP ( $P < 0.05$ ) and MBN ( $P < 0.05$ ). In addition, the relative abundance of Gemmatimonadetes and Nitrospirae was positively correlated with AN and CAT but negatively correlated with pH. We also observed that there was no significant correlation between Bacteroidetes, Chloroflexi and Verrucomicrobia with soil biochemical properties.



**Figure 8.** The relationship between soil biochemical properties (black arrows) and bacterial communities that ranked within the top 10 at phylum level (red arrows) using redundancy analysis (RDA).

## 4. Discussion

### 4.1. Effect of Vegetation Restoration on Soil Biochemical Properties

Reclamation on reconstructed soil of dumps in mining areas significantly improved soil biochemical properties compared with the UL, confirming the findings of previous studies. For instance, Wang et al. [33] reported that vegetation restoration plays a major role in promoting and recovering the fragile ecological environment, especially in mining areas on the Loess Plateau. Yang et al. [34]

observed that reclamation can significantly improve soil nutrient content. In this study, different vegetation types and coverage of the four sampling sites led to differences in soil nutrient contents and enzyme activities. These differences significantly affected soil MBC and MBN. As shown in Figure 2, the BL was superior in the majority of properties to the other three sampling sites, which is consistent with previous findings [35]. Under the influence of a series of physical and biological processes, the soil under shrubs in arid and semi-arid areas has a higher soil nutrient content, known as the “fertility islands effect” [36,37]. Soil available nutrients are closely related to soil enzyme activities because soil enzyme activities directly affect soil nutrient mineralization and the soil nutrient cycle changes with the change of mineralization [34]. Land use changes, such as vegetation restoration, may potentially change the abundance of ecological resources by changing their dynamics and conversion rate [38]. Compared with the UL site, artificial vegetation restoration can improve the activities of CAT, URE, ALP and SUC (Figure 2). Activities of ALP and URE were higher in the BL than the other sites, indicating that this vegetation restoration type would increase both AP and AN content. Activities of CAT and SUC were higher under the GL and FL than the other sites, respectively. In total, this study showed a faster increase in soil quality under artificial vegetation restoration than under the UL. The contents of soil MBC and MBN were significantly different among the four sampling sites ( $P < 0.05$ ) and were highest in the BL and lowest in the UL. This could be attributed to the root of shrubs dominated by legumes which excrete a high amount of sugars, amino acids and other low-molecular-weight organic compounds. These compounds can positively affect microbial growth [39]. Shrubs dominated by legumes could be recommended for reclamation of reconstructed soil in mining areas on the Loess Plateau.

#### 4.2. Effect of Vegetation Restoration on Bacterial Communities

The OTUs, bacterial community richness (ACE and Chao1) and diversity (Shannon and Simpson) indices were significantly affected by reclamation ( $P < 0.05$ ) according to the results shown in Table 1. Generally, soil moisture is an important factor regulating soil microbial biomass and microbial community richness [40]. In this study, the soil moisture in the UL was the lowest (Figure 2,  $P < 0.05$ ), which may result in the soil microbial biomass and microbial  $\alpha$ -diversity index of unreclaimed land being lower than that of vegetation restoration (Figure 2, Table 1). However, the bacterial community structure remained relatively stable among the four sampling sites (Figure 5, Figure 6 and Supplementary Materials Table S2). Preceding studies have shown that land-use history is more important than soil properties in influencing microbial community structure [41]. On account of the dramatic effects of agricultural activities, the soil microbial community of tallgrass prairie has not fully recovered even after more than 30 years at the Konza Prairie Biological Station, USA [42]. In the current research, the vegetation restoration period was 18–20 years, which was not sufficient to change the composition of the microbial community.

Proteobacteria, Actinobacteria and Acidobacteria were identified as the major phyla in this study region, which is consistent with previous research [43–45] and indicates that these phyla are not exclusive to the arid and semi-arid region of the Loess Plateau. The relative abundance of Proteobacteria in the FL and BL was higher than GL and UL, while that in the UL was lowest. Studies have shown that Proteobacteria was dominant in soil bacterial communities of forest [46], shrub [47] and grassland soils [10]. Proteobacteria in the current study area mainly included Alphaproteobacteria and Betaproteobacteria in two sub-groups (Supplementary Materials Figure S2). Dedysh et al. [48] reported that Alphaproteobacteria and Betaproteobacteria include N-fixing bacteria that can coexist with plants, hence, relatively higher abundance of Proteobacteria could be conducive to promoting N fixation ability in soil. In addition, Janssen [49] suggested that Alphaproteobacteria might be related to the high nutrient content in the soil. In general, the relative abundance of Actinobacteria showed a decreasing trend with vegetation restoration [43]. Our results (Figure 3) similarly suggest that the abundance of Actinobacteria had a negative response to reclamation and vegetation restoration. Naether et al. [50] reported that the highest relative abundance of Acidobacteria was found in soils with the lowest pH; the relative abundance of Acidobacteria in the current study ranged from 8.4% to

12.8%, which was much lower than that of acid mine drainage barrens in Central Pennsylvania, USA (14.14%–30.85%) [51], Dinghushan forest (53.3%–67.8%) and Sanjiang plain (53%) in acid soil areas of China [52,53]. This may be because the alkaline environment of the experimental area inhibited the growth of Acidobacteria and promoted the growth of alkalophilic bacteria, increasing the competition pressure between bacteria in soil and reducing the relative abundance of the Acidobacteria.

The results of this study indicate that vegetation restoration had an effect on the relative abundance and diversity of soil bacteria.

#### 4.3. Response of Soil Bacterial Communities to Soil Biochemical Properties

In this study, the soil bacterial communities among three vegetation restoration types and UL remained relatively consistent. This result is similar to the findings of Cui et al. [9] that there were no significant changes in soil bacterial communities under three restoration types in the semiarid region of the Loess Plateau. In contrast, Zhang et al. [54] showed that during the vegetation restoration process on the Loess Plateau, the dominant bacterial flora changed, which was reflected in the decrease of the abundance of Acidobacteria and the increase of the abundance of Proteobacteria. The inconsistent results may be due to the different ages of vegetation restoration; the vegetation restoration period of Zhang et al. [54] was 30 years, which was longer than that of our study (18–20 years). Owing to the lagging response of soil microbes to environmental changes [55], the true situation of soil bacterial communities could not be fully reflected even after 18–20 years of vegetation restoration.

RDA (Figure 8) and Pearson's correlation coefficients (Supplementary Materials Table S3) showed the relationship between relative abundance of dominant bacterial communities and soil biochemical properties, indicating that variation in soil bacterial communities has little response to the changes of soil biochemical properties. The dominant bacterial communities (Proteobacteria, Actinobacteria and Acidobacteria) were significantly correlated with only some of the soil biochemical properties. In this study, Proteobacteria was positively correlated with all biochemical indicators except AP and its relative abundance showed an increasing trend from UL to vegetated restoration land (Figure 3), probably due to increased C and N content. Resource heterogeneity helps to maintain high microbial diversity and coexistence. In general, vegetation provides resource heterogeneity for soil microbial community through multiple factors such as litter decomposition and root system secretion. Therefore, higher plant diversity leads to better soil microbial community diversity and coexistence; in addition, anthropogenic managed activities, such as the application of nutrients, also increase the heterogeneity of resources for soil microbes [38]. The abundance of this phylum was positively correlated with the available C and N in the soil [56,57]. In artificial vegetation restoration areas, in addition to accumulation of C and N by the decomposition of litter and root exudates, artificial fertilization also promoted the growth of Proteobacteria. The Actinobacteria in this study presented the opposite results to the Proteobacteria, with a higher abundance in the UL compared with the vegetated restoration areas. The Actinobacteria negatively correlated with all biochemical indicators except AP, indicating that this phylum showed a negative response to reclamation. Acidobacteria were positively correlated with most biochemical properties and had a positive response to vegetation restoration (Figure 3, Figure 8). This could be because the Acidobacteria may be suitable for a weak acid environment and organic acids secreted by plant roots can reduce the pH value of soil, which is conducive to the growth of Acidobacteria. This is supported by the negative correlation between Acidobacteria and pH (Figure 8 and Supplementary Material Table S3).

In summary, the relative abundance of most bacterial communities was not significantly correlated with soil biochemical properties ( $P < 0.05$ ), indicating that the bacterial community structure had no significant response to changes in soil biochemical properties, despite 18–20 years of vegetation restoration.

This study is only a case of one locality, the results contribute to general understanding of the effects of the vegetation restoration, however, they cannot be considered general due to particular local conditions and the high complexity of soil microbial communities. In the future, a series of related

studies should be conducted in different regions and soil types to further explore the relationship between soil, vegetation and microbes.

## 5. Conclusions

The findings of this study suggest that vegetation restoration on the reconstructed soil in the mining area of the Loess Plateau can significantly improve OTUs, bacterial community richness (ACE and Chao1) and diversity (Shannon and Simpson) indices and some selected soil biochemical properties. However, the structure of the soil bacterial community did not change significantly, even though the region has undergone nearly 20 years of vegetation restoration. Results of RDA and Pearson's correlation coefficient revealed that the response of bacterial community to biochemical properties was not obvious and the dominant phyla were Proteobacteria, Actinobacteria and Acidobacteria. Since the BL soil had better biochemical properties, bacterial richness and diversity, it was considered the optimum restoration type for this area. In general, this study offered some information on the response of soil microbes to environmental changes of mining areas in arid and semi-arid regions of China and demonstrated the advantages of high-throughput sequencing in the study of soil microbial communities. In the future, it is necessary to study the soil biochemical properties and microbial community characteristics in the vegetation restoration area of the Loess Plateau mining area long term and under different periods of restoration.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2071-1050/11/8/2295/s1>.

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