The influence of transplanted trees on soil microbial diversity in areas affected by coal mining subsidence of the Loess Plateau in China

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Abstract: Soil microbial diversity in areas affected by coal mining subsidence is closely associated with vegetation restoration. In this study, we compared the diversity of soil bacteria and fungi under different vegetation restoration modes in the subsidized Daliuta coal mining region in western China. The dominant bacteria, *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, and *Acidobacteria,* were found at abundances of 29.43–34.68%, 15.87–24.75%, 13.09–19%, and 12.06–15.36%, respectively. The dominant fungi, *Ascomycetes* and *Zygomycetes,* had abundances of 23.96–71.08% and 10.42–56.26%, respectively. The diversity indices (S_{obs}, Shannon, and Chao1) of the rhizosphere soil bacteria and fungi were significantly lower in the primary *Stipa breviflora* phytocommunity than in the

phytocommunities of transplanted trees. Among physicochemical soil parameters, total nitrogen (TN), total phosphorus (TP), water content (WC), and pH affected soil bacterial diversity, and available phosphorus (AP) and TN affected bacterial community structure the most. Furthermore, WC affected soil fungal diversity, whereas TP and TN mostly affected the fungal community structure. However, edaphic factors did not uniformly affect all microbial groups. Although TN, WC, and AP significantly influenced the species richness of *Actinobacteria* and *Proteobacteria* ($p<0.05$), TP was significantly negatively correlated with species richness in *Acomycota, Basidiomycota,* and *Zygomycota* ($p<0.05$). However, TN influenced the species richness of *Zygomycota* ($p<0.05$) . Furthermore, we found that the rhizosphere microbial diversity of the CK phytocommunity differed from that of the transplanted tree communities in the study area; i.e., the transplanted trees promoted soil microbial diversity in phytocommunities, and moreover, different edaphic factors varied in their effect on the community composition of rhizosphere bacteria and fungi. We will continue to monitor the soil microbial diversity in the study area with the goal to provide guidance for environmental remediation of areas affected by coal mining subsidence.

Keywords: transplanted trees; soil microbial diversity; coal mining subsidence; the Loess Plateau

1. Introduction

China's major coal deposits are located in the ecologically vulnerable arid and semiarid areas in the west of the country. Shendong, which has approximately 25% of China's coal reserves, is a large coal mining region in western China. The region is arid, poor in biodiversity and vegetation cover, and exposed to severe soil desertification [1]. Large-scale coal mining usually causes various ecological problems, including ground subsidence, ground fractures, vegetation deterioration, and soil pollution [2]. The Shendong coal mining region faces the same problems, as well as substantial challenges in environmental governance. The countermeasures implemented in Shendong against ground subsidence and fractures include afforestation projects, applying soil conditioners, and adding functional microorganisms [3], which are all intended to improve the phytocommunities and landscape. However, owing to the regional environment, including climatic conditions, transplanted trees often have a withered appearance and a low survival rate.

To our knowledge, no in-depth studies have been conducted to investigate the interactions between transplanted trees and soil in the areas affected by mining subsidence, including on the effects of the transplanted trees on soil microbial diversity [4,5]. As a major biotic soil component, microbial communities affect not only the growth and development of plants and supply of soil nutrients but also indicate land disturbance, along with changes in soil microenvironments and land utilization patterns [6,7]. Farming affects the diversity of soil bacteria and archaea. The phylum-level diversity of bacteria is higher in forests than farmlands but species-level diversity is lower in forests than in farmlands, whereas archaeal diversity is far lower in forests than in farmlands [8].

Coal mining subsidence destroys aboveground vegetation (e.g., by tearing and pulling root systems) and soil structure (e.g., by fractures and collapses), considerably disturbing the diversity and density of soil microbial communities [2,4]. In grassland vegetation restoration projects, the biomass, community structure, and ecological functions of soil microbes vary with the planting pattern [9,10]. Changes in microbial diversity affect the concentration of mineral elements and enzymes in the soil [11]. The characteristics of soil microbial communities correlate significantly with the physicochemical properties of soil, such as soil bulk density and the concentration of nitrogen sequestered as nitrate and soil minerals. For instance, although the quantity of soil microbes involved

in nitrogen transformation (e.g., ammonifying, nitrifying, and denitrifying bacteria) increases significantly during vegetation restoration, their density varies widely among phytocommunities [12]. Thus, the response of soil microbial communities varies with the pattern of vegetation restoration, and the biomass of soil microbes correlates significantly with the total nitrogen (TN) and total carbon (TC) in soil [3].

Vegetation restoration is an important method for improving biodiversity and ecological functions in areas affected by coal mining subsidence ,because it improves soil properties and promotes the restoration of soil microbial communities. This study focused on the areas affected by coal mining subsidence in the Loess Plateau of western China, including the communities of transplanted trees. We assessed the differences between the *Stipa breviflora* phytocommunity and transplanted tree communities (e.g., *Populus simonii*, *Caragana korshinskii*, *Hippophae rhamnoides*, *Pinus tabuliformis*, *Amygdalus pedunculata*, and *Armeniaca sibirica*) in terms of bacterial and fungal diversity and community structure of rhizosphere soil and analyzed their correlation with edaphic factors. We explored the effects of transplanted trees on soil microbial communities and regional vegetation restoration in areas affected by coal mining subsidence to provide guidance for vegetation restoration projects and environmental governance in such areas.

2. Materials and methods

2.1. Study sites

Located in the Shendong Coal Mine of Daliuta Town, Shenmu County, Shaanxi Province, China, the study area lies at the juncture of the Loess Plateau and Mu Us Desert and stretches from 39°16′9.97″N to 39°16′58.28″N, and from 110°18′57.19″E to 110°19′20.13″E (Figure 1). The altitude ranges from 1,216 to 1,265 m, the average annual precipitation is 437.5 mm, and the precipitation from July to September accounts for at least 60% of annual precipitation. The annual average sunshine is 2,876 hours, the annual average temperature is 8.4 °C, the extreme minimum temperature is –28.1 °C, and the extreme maximum temperature is 38.9 °C. The annual average frost-free period is 153 days. The vegetation in the study area represents a typical prairie of the warm temperate zone; specifically, the prairie community dominated by *Stipa breviflora* of the grass family grows on the chestnut or loess soil of loess hills. Soil erosion caused by water and soil loss is severe, and vegetation

is sparse. Large-scale coal mining has produced large subsidence areas and an abundance of fractures of varying sizes (3–50 cm in width); the maximum difference in fracture height is 1.5 m. Afforestation has been carried out extensively in the subsidence areas. Coal mining subsidence occurred in the study area in 2011, and an ecological governance project was implemented in this area in 2012.

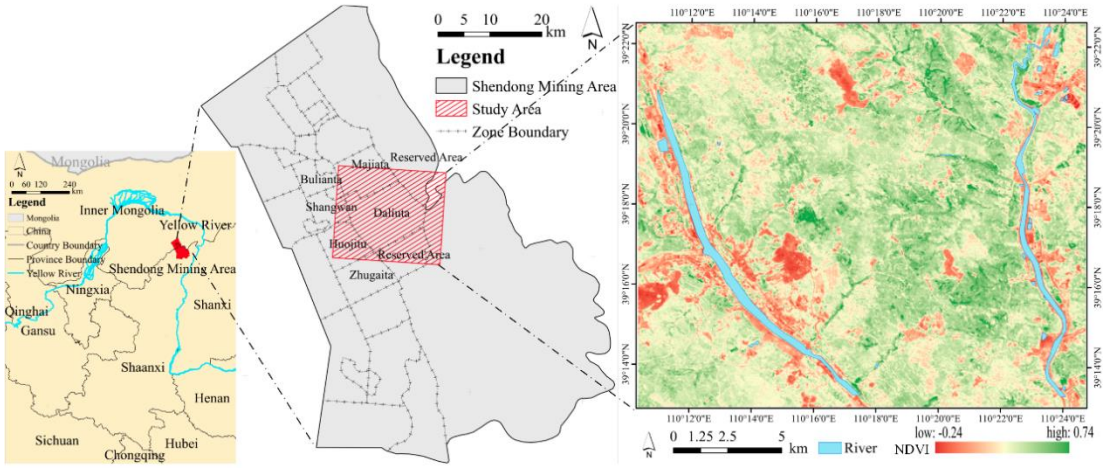


Figure 1. Location and landscape of the study area. Maps of the study area in the Shanxi Province and Inner Mongolia, China, along with the location of the border of the Shendong arid coal mining area and Daliuta mine (upper panels). Examples of the vegetation and landscape of the study area (lower panels).

2.2. Sampling and soil characterization

In September 2017, we selected the primary *S. breviflora* community in the mining subsidence area of the Shendong coal mine, along with communities of transplanted *P. simonii*, *C. korshinskii*, *H. rhamnoides*, *P. tabuliformis*, *A. pedunculata*, and *A. sibirica*, as sample areas for our study, which were referred to as CK, R1, R2, R3, R4, R5, and R6, respectively. In each sample area, a randomly placed quadrat of 10 m × 10 m enclosed five 1 m × 1 m quadrats placed diagonally at its opposite angles and

center. In each quadrat, surface soil (~2 cm in depth) was removed, and soil samples from 5 to 30 cm in depth were collected at five points. Then the soil samples from the five quadrats were combined as one representative soil sample of the community. For each community of transplanted trees, five rhizosphere soil samples from 0 to 20 cm in depth were collected and combined [13]. Each 1-kg soil sample was sealed in an aseptic Ziplock bag, marked, and analyzed in a laboratory [14,15].

Within 48 hours, each soil sample was divided into two parts. One part was filtered through a 2-mm sieve and used to determine the physicochemical properties of the soil [15]. The other part was stored in a freezer at −80 °C until further processing [16] in preparation for a nested PCR-DGGE (denaturing gradient gel electrophoresis) analysis to assess bacterial and fungal diversity. In accordance with the seven sampling sites, there were seven corresponding soil samples that were tested in triplicate (21 soil samples in total).

2.3. Analysis of physicochemical soil parameters

The soil water content was determined using the oven drying method [16], and the soil pH was measured with a Mettler Toledo desk-type pH meter (Five Easy Plus, Mettler Toledo, China) [17]. The soil concentrations of ammonium nitrogen, rapidly available phosphorus, and available potassium were determined with a soil analyzer (TPY-6pc, Top Instrument, Zhejiang, China); the TN, TP (total phosphorus), and TC values were determined using an elemental analyzer (Vario EL III, Germany) [16].

2.4. DNA extraction, PCR amplification, and sequencing

Aliquots of 0.5 g of fresh sample material were ground in a mortar under liquid nitrogen. The DNA of the rhizosphere soil samples was extracted using the E.Z.N.A.® DNA reagent kit (DP336, Omega BioTek, Norcross, GA, U.S.). Extracted DNA samples were subjected to gel electrophoresis (sepharose gel, 1%) and evaluated for purity and concentration. The DNA samples were stored at −80 °C until further analysis by PCR.

To analyze the composition of microbial communities by PCR-DGGE, primers were designed to target bacteria and fungi for sequencing analysis using the Illumina MiSeq high-throughput sequencing technology (Illumina, San Diego, CA). The primers targeting the V3 to V4 region of the

16S rDNA of soil bacteria were 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), which were used to amplify a 468-bp fragment for analysis using the PE30 sequencing method. For soil fungi, the primers targeting the ITS1 to ITS2 region of the rRNA gene were ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'), which were used to amplify fragments of ~300 bp for analysis using the PE300 sequencing method. Specific primers with barcodes targeting the amplified areas were synthesized and used in 454 pyrosequencing to completely cover entire bacterial and fungal communities.

After a successful pre-experiment, 20-μL PCR mixes were prepared to amplify each sample, including its replicates. Each reaction consisted of 100–200 ng DNA, 10 μL 2× Easy Taq PCR SuperMix polymerase (TransGen Biotech; containing 1.25 U Ex Taq, 0.4 mM dNTP, and 4 mM Mg^{2+}), 0.4 μM primers, and ddH_2O. The PCR conditions included an initial denaturation at 95 °C for 3 min, followed by the denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s. A total of 27 cycles were performed for soil bacteria and 35 cycles for soil fungi. PCR products were stored in a freezer.

For further processing, three PCR replicates of each sample were mixed and subjected to electrophoresis (sepharose gel, 2%). The frozen samples were melted on the ice, then mixed completely, and centrifuged. Aliquots of 2 μL of the mixed samples were evaluated by gel electrophoresis using the following conditions: sepharose gel, 2%; constant voltage, 120 V; and running time, 30 min. The amplified PCR fragments were purified with beads to generate a sequencing library. Using the Illumina MiSeq 2 × 300 bp paired-end (PE) method, the library was sequenced by Shanghai Majorbio Bio-Pharm Technology Co., Ltd.

2.5. Statistical analysis

The raw sequencing data included some invalid data that had to be removed. For the paired-end data obtained through MiSeq sequencing, the samples were distinguished and sorted according to the barcode information. Then the paired-end data were merged and spliced into tags according to the overlapping alignments. Subsequently, the spliced data were filtered. Finally, quality control and analysis (e.g., Q20 and Q30) were performed [18]. The validated data (https://www.i-sanger.com/)

were subjected to a clustering analysis to generate operational taxonomic units (OTUs) needed to assign species. Specifically, the OTUs derived from this clustering analysis using taxonomic databases (e.g., RDP, Greengenes, and NCBI resource database) were subjected to species categorization and subsequent statistical analysis.

Based on the results of OTU analysis, the S_{obs}, Shannon, and Chao1 indices were analyzed. The species abundance and diversity using Mothur (version v.1.30.1) software for index calculation [19].On the software platform Usearch (version 7.0; http://drive5.com/uparse/), single-factor ANOVA and Pearson correlation coefficient analysis (e.g., the differences of soil properties and bacterial and fungal richness) were performed using SPSS 22.0. Using the principal component analysis (PCA) method, the Bray-Curtis distance was subjected to a dimensionality reduction analysis to derive community structures. Furthermore, the effects of various environmental factors on bacterial and fungal community structures were analyzed by redundancy analysis. To calculate the Bray-Curtis distance, along with the matrix dimensionality reduction and redundancy analysis (RDA), the "vegan" package was used for statistical analysis and drawing in R 3.3.3.

3. Results

3.1. Microbial richness and diversity

At a similarity level of 0.03 for bacterial and fungal OTUs, the representative sequences of these OTUs with a similarity of 97–99% were subjected to a taxonomic analysis using the RDP classifier Bayesian algorithm. The values of the coverage index were equal to or greater than 0.97. At the similarity level of 0.03, the sequencing results indicated the status of the bacteria and fungi present in a soil sample without significant differences.

Table 1. Microbial richness and diversity indices of rhizosphere soil samples.

Site	S_{obs}	Shannon index	Chao1 index	Coverage
		Bacteria		
R1	2473 ± 18.01e	6.59 ± 0.02d	3318.83 ± 18.21c	0.97
R2	2622.33 ± 8.99d	6.72 ± 0.02c	3238.86 ± 53.91cd	0.97
R3	2459.33 ± 34.12e	6.62 ± 0.02d	3105.81 ± 83.88d	0.97
R4	2884 ± 27.79b	6.91 ± 0.02a	3729.58 ± 31.42a	0.97
R5	2963 ± 24.01a	6.83 ± 0.03b	3862.05 ± 28.96a	0.97
R6	2715 ± 27.1c	6.70 ± 0.03c	3533 ± 18.24b	0.97
CK	2176.33 ± 24.13f	6.37 ± 0.02e	2891.71 ± 54.22e	0.97
		Fungi		
R1	386.33 ± 40.48b	3.42 ± 0.15ab	485.40 ± 52.33b	0.99
R2	488.33 ± 36.84a	3.33 ± 0.51ab	602.27 ± 37.7a	0.99
R3	295.33 ± 10.81c	3.23 ± 0.1ab	331.70 ± 20.36c	0.99
R4	444.33 ± 16.25ab	4.18 ± 0.09a	487.95 ± 11.82b	0.99
R5	461.67 ± 42.44ab	2.85 ± 0.57b	535.70 ± 39.45ab	0.99
R6	453 ± 14.01ab	3.72 ± 0.13ab	520.78 ± 15.17ab	0.99
CK	255.33 ± 21.07c	2.74 ± 0.12b	311.41 ± 16.87c	0.99

Note: Observed species numbers are S_{obs}. The numerical values are average values ± standard deviations, and small letters indicate the significant differences between different sampling areas ($p < 0.05$).

Table 1 describes the high-throughput sequencing results of the rhizosphere soil microbes associated with the phytocommunities dominated by CK and the transplanted trees in the areas affected by coal mining subsidence.

The analysis of bacterial diversity indices showed that the S_{obs}, Shannon, and Chao1 indices, were higher for the rhizosphere bacteria of the transplanted tree phytocommunities than for those of the primary CK phytocommunity. The diversity levels sorted in descending order according to the Chao1 index were R5 > R4 > R6 > R1 > R2 > R3 > CK; and according to the Shannon index, R4 > R5 > R2 > R6 > R3 > R1 > CK. Thus, the richness and diversity of the rhizosphere bacteria of the transplanted trees phytocommunities were significantly higher than those of the CK phytocommunity ($p < 0.05$).

Specifically, the rhizosphere soil of R4 had the highest bacterial richness and diversity.

The fungal diversity differed among the phytocommunities according to the Chao1 index, sorted in descending order, R2 > R5 > R6 > R4 > R1 > R3 > CK, and according to the Shannon index, sorted in descending order, R4 > R6 > R1 > R2 > R3 > R5 > CK. The index values indicated that the richness and diversity of the rhizosphere fungi of the transplanted trees phytocommunities were significantly higher than those of the primary CK phytocommunity. Specifically, the rhizosphere soil layer of R2 had the highest fungal richness, and the rhizosphere soil layer of R4 had the highest fungal diversity.

Overall, the transplanted trees in the areas affected by coal mining subsidence increased the bacterial and fungal richness and diversity of the rhizosphere soil layer. Interestingly, the rhizosphere soil of the R4 phytocommunity had the highest microbial diversity.

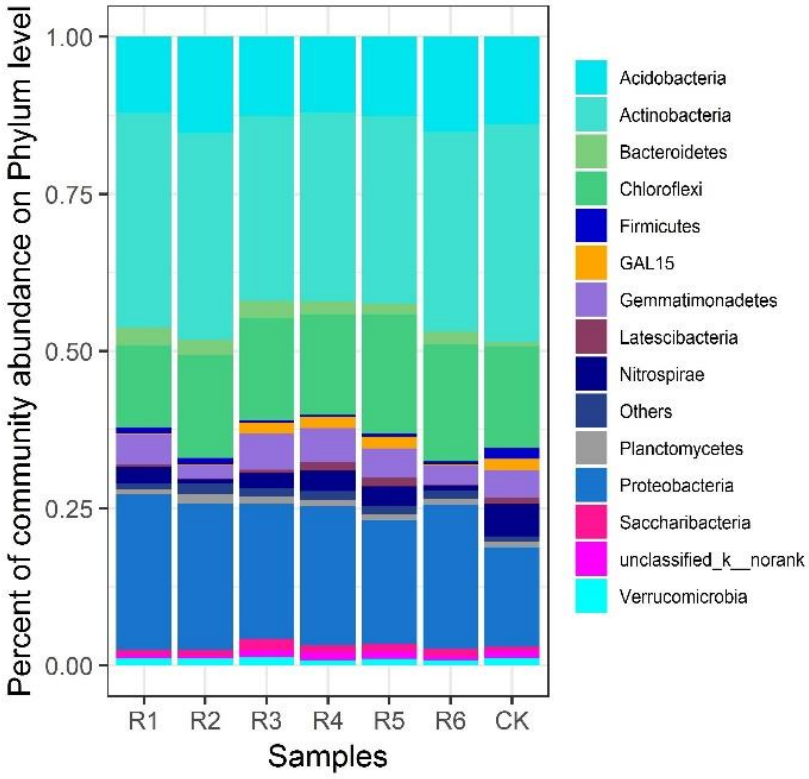
3.2. Differences in soil microbial communities

The PE reads obtained by MiSeq high-throughput sequencing were first spliced according to the overlap alignments. After control sequence analysis and data filtering were performed, a total of 1,207,639 sequences were generated from 21 bacterial samples; a total of 528,372,652 bp were sequenced, and the average sequence length was 437.55 bp. A total of 1,177,106 sequences were generated from 21 fungal samples, the fungal data included a total of 321,597,792 bp, and the average sequence length was 273.7 bp (https://www.i-sanger.com/).

3.3. Soil microbe community structure

In the CK phytocommunity of the areas affected by coal mining subsidence, the proportions among the dominating bacterial groups in the rhizosphere were as follows: *Actinobacteria* (34.68%) > *Chloroflexi* (16.16%) > *Proteobacteria* (15.87%) > *Acidobacteria* (13.9%), as shown in Figure 2. In the rhizosphere of the R1 phytocommunity, the abundance of the major bacterial groups were as follows: *Actinobacteria* (34.17%) > *Proteobacteria* (24.76%) > *Chloroflexi* (13.09%) > *Acidobacteria* (12.12%). In the R2 phytocommunity rhizosphere, the proportions of the dominating bacterial groups were as follows: *Actinobacteria* (32.84%) > *Proteobacteria* (23.31%) > *Chloroflexi* (16.34%) > *Acidobacteria* (15.36%). In the phytocommunity of R3, the abundance of major rhizosphere bacterial groups were as follows: *Actinobacteria* (29.43%) > *Proteobacteria* (21.45%) > *Chloroflexi* (16.3%) >

229 *Acidobacteria* (12.61%). In the rhizosphere of the R4 phytocommunity, the proportions of the
230 dominating bacterial groups were as follows: *Actinobacteria* (30.03%) > *Proteobacteria* (22.11%) >
231 *Chloroflexi* (16.06%) > *Acidobacteria* (12.06%). In the phytocommunity of R5, the abundance of
232 major rhizosphere bacteria groups were as follows: *Actinobacteria* (29.88%) > *Proteobacteria*
233 (19.71%) > *Chloroflexi* (19.00%) > *Acidobacteria* (12.57%). In the R6 phytocommunity rhizosphere,
234 the proportions among the dominating bacterial groups were as follows: *Actinobacteria* (31.74%) >
235 *Proteobacteria* (22.94%) > *Chloroflexi* (18.61%) > *Acidobacteria* (15.14%).



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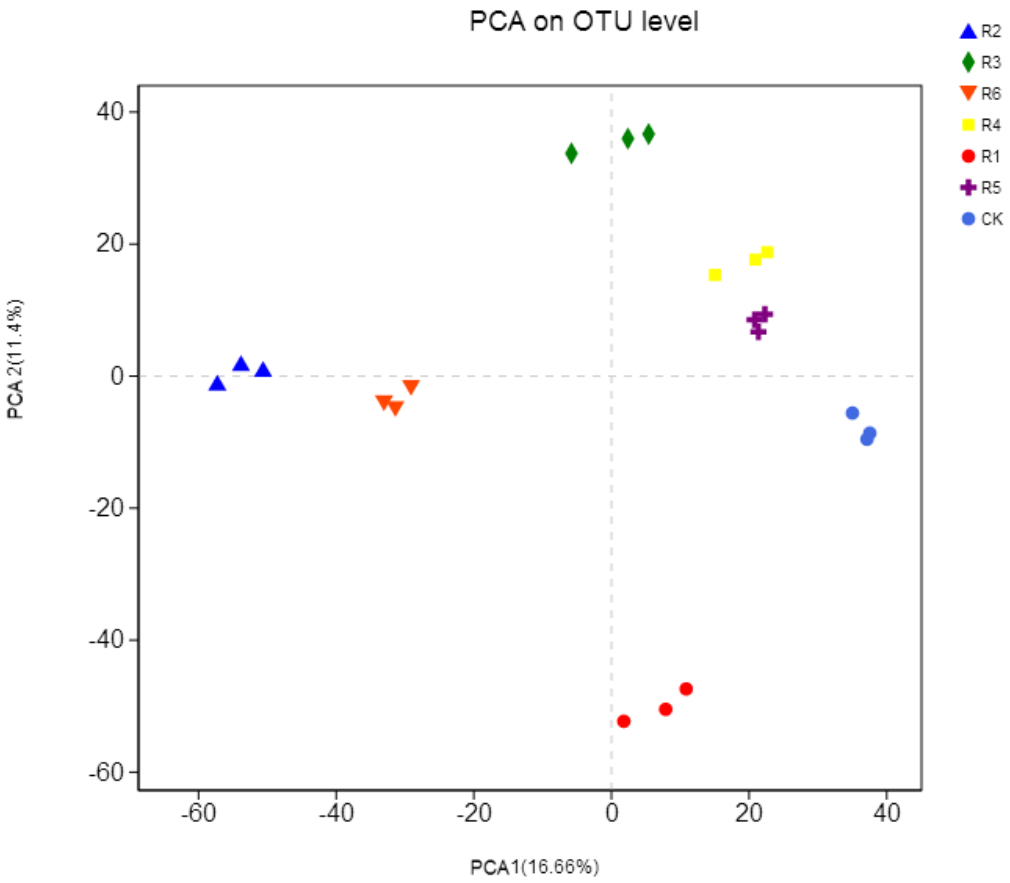
237 **Figure 2.** Percent of community abundance on Phylum level in the rhizosphere soil of phytocommunities in mining
238 subsidence areas.

239 The PCA results indicated that, in areas affected by coal mining subsidence, the bacterial
240 community parameters of the rhizosphere soil differed between the CK phytocommunity and the
241 phytocommunities of transplanted trees (Figure 3). Specifically, the CK phytocommunity was the
242 closest to the phytocommunities of R5 and R4 but farthest from the R2 phytocommunity. Thus, the
243 most significant difference in the rhizosphere bacterial community composition existed between the
244 CK and R2 phytocommunities, whereas the difference was less significant among the

245 phytocommunities of CK, R5, and R4.



246

247 **Figure 3.** Principal component analysis (PCA) of the rhizosphere soil bacteria of phytocommunities in mining

248 subsidence areas.

249 In the *CK* phytocommunity, the proportions among the dominating rhizosphere fungal groups

250 were as follows: *Zygomycota* (56.15%) > *Ascomycota* (24%) > *Unclassified_k_Fungi* (17.82%) >

251 *Basidiomycota* (2%) (Figure 4). In the R1 phytocommunity, the abundance of the major rhizosphere

252 fungi groups were as follows: *Ascomycota* (44.19%) > *Zygomycota* (23.43%) > *Unclassified_k_Fungi*

253 (8.95%) > *Basidiomycota* (16%). In the R2 phytocommunity rhizosphere, the proportions among

254 dominating fungal groups were as follows: *Ascomycota* (64.45%) > *Zygomycota* (7.63%) >

255 *Unclassified_k_Fungi* (24.54%) > *Basidiomycota* (2.87%). In the R3 phytocommunity rhizosphere,

256 the proportions of major fungal groups were as follows: *Ascomycota* (58.6%) > *Zygomycota* (15.33%) >

257 *Unclassified_k_Fungi* (13.53%) > *Basidiomycota* (11.76%). In the rhizosphere of the R4

258 phytocommunity, the abundance of the dominating fungal groups were as follows: *Ascomycota*

(59.64%) > *Zygomycota* (13.12%) > *Unclassified_k_Fungi* (10.84%) > *Basidiomycota* (15.88%). In the R5 phytocommunity rhizosphere, the proportions of major fungal groups were as follows: *Ascomycota* (61.5%) > *Zygomycota* (10.43%) > *Unclassified_k_Fungi* (2.37%) > *Basidiomycota* (25.6%). In the R6 phytocommunity, the proportions of major rhizosphere fungi groups were as follows: *Ascomycota* (70.46%) > *Zygomycota* (19.9%) > *Unclassified_k_Fungi* (4.08%) > *Basidiomycota* (5.54%).

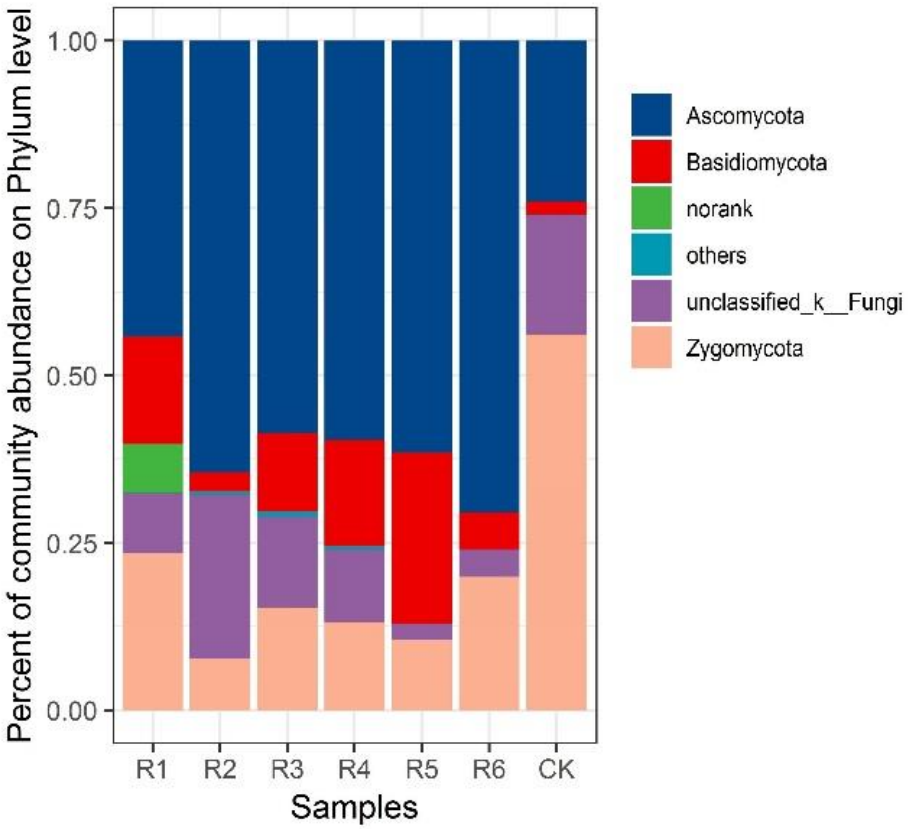


Figure 4. Percent of community abundance on Phylum level in the rhizosphere soil layer of phytocommunities in mining subsidence areas.

The PCA results (Figure 5) demonstrated that in areas affected by coal mining subsidence, the rhizosphere fungi of the CK phytocommunity were closest to those of the R3 phytocommunity but relatively far from the rhizosphere fungi of the other phytocommunities of transplanted trees. Thus, the rhizosphere fungi community composition of the CK phytocommunity differed from the other phytocommunities of transplanted trees; specifically, it slightly varied from the R3 phytocommunity

but significantly differed from the other types of transplanted trees.

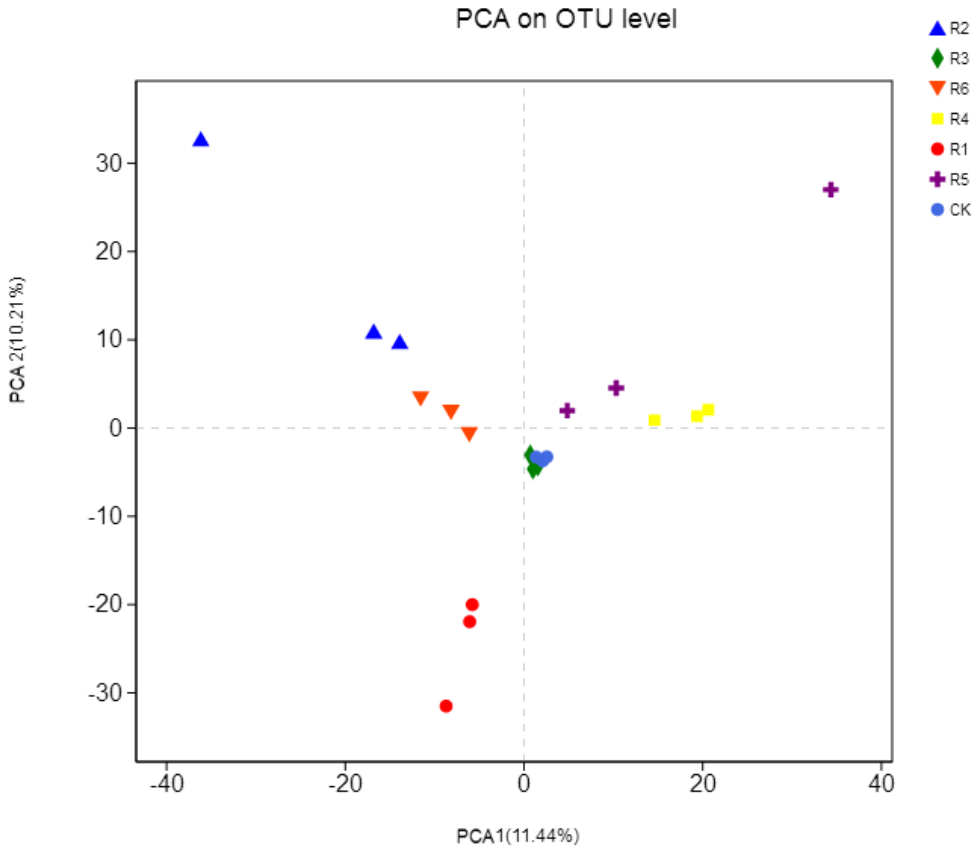


Figure 5. Principal component analysis (PCA) of rhizosphere soil fungi of phytocommunities in mining subsidence areas.

3.4. Effects of edaphic factors on bacterial diversity

The soil TN exhibited a highly significant positive correlation with the S_{obs}, Shannon, and Chao1 indices of soil bacteria ($p < 0.01$), whereas the soil TP had a significant negative correlation with the S_{obs} and Chao1indices of soil bacteria ($p < 0.05$). The soil WC had a significant negative correlation with the Chao1index of soil bacteria ($p < 0.05$), and the soil pH had a significant positive correlation with the respective Shannon index ($p < 0.05$) (Table 2).

Table 2. Correlation between bacterial diversity indices and physicochemical soil parameters.

Soil parameter	S_{obs} index	Shannon index	Chao1 index
WC	−0.309	−0.318	−0.472*

pH	0.339	0.455*	0.222
NH_4-N	0.086	0.234	−0.039
AP	−0.023	0.016	−0.008
AK	−0.297	−0.369	−0.248
TN	0.648**	0.683**	0.557**
TC	0.038	0.082	−0.083
TP	−0.486*	−0.360	−0.461*

WC, soil water content; NH_4-N, ammonium nitrogen; AP, available phosphorus; AK, available potassium; TN, total nitrogen; TC, total carbon; TP, total phosphorus; Spearman correlation analysis: * P <0.05; ** P <0.01.

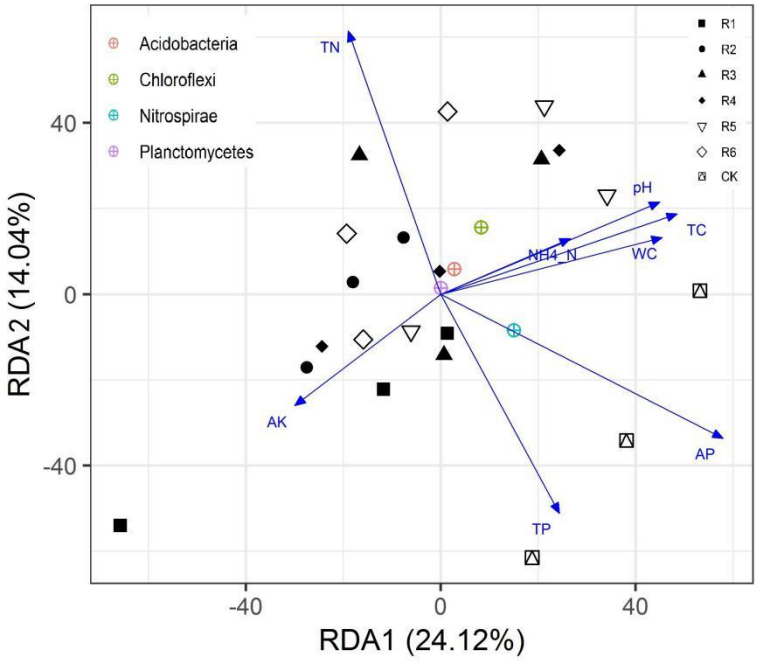


Figure 6. Redundancy analysis (RDA) for rhizosphere soil bacteria and edaphic factors in artificial phytocommunities.

The results of the RDA for physicochemical soil factors and bacterial communities are shown in Figure 6. RDA1 and RDA2 accounted for 24.12% and 14.04% of the bacterial community composition in soil, respectively. Among the eight soil parameters, AP and TN affected the soil bacteria most significantly, but they had opposite effects on the bacterial community structure.

Table 3. Correlation between bacterial species abundance indices and physicochemical soil parameters.

Soil parameter	*Actinobacteria*	*Proteobacteria*	*Chloroflexi*	*Acidobacteria*

WC	−0.048	−0.516*	0.261	0.053
pH	−0.382	−0.383	0.212	−0.216
NH_4-N	−0.185	−0.104	0.147	0.051
AP	0.033	−0.518*	−0.017	−0.144
AK	0.19	0.071	−0.194	0.297
TN	−0.509*	0.111	0.206	−0.087
TC	−0.364	−0.42	0.231	−0.162
TP	0.411	−0.217	−0.363	0.056

WC, soil water content; NH_4-N, ammonium nitrogen; AP, available phosphorus; AK, available potassium; TN, total nitrogen; TC, total carbon; TP, total phosphorus; Spearman correlation analysis: * $P<0.05$; ** $P<0.01$.

The effects of physicochemical soil parameters on the richness of bacterial communities were as follows. TN was significantly negatively correlated with the richness of *Actinobacteria* ($p<0.05$), and WC and AP were significantly negatively correlated with the richness of *Proteobacteria* ($p<0.05$) (Table 3). Thus, AP was the primary soil environment factor that affected the bacterial community structure in the rhizosphere soil layer of the CK phytocommunity, and TN affected the rhizosphere bacteria of the phytocommunities dominated by R2, R4, and R6. TN affected the richness of *Actinobacteria*, but WC and AP had an effect on the richness of *Proteobacteria*.

3.5. Effects of edaphic factors on fungal diversity

The analysis of correlations between edaphic factors and the S_{obs}, Shannon, and Chao1 indices of rhizosphere fungi revealed that WC had a significant negative correlation ($p<0.05$) with the S_{obs} index and a highly significant negative correlation ($p<0.01$) with the Shannon index. Thus, WC affected fungal diversity most significantly (Table 4).

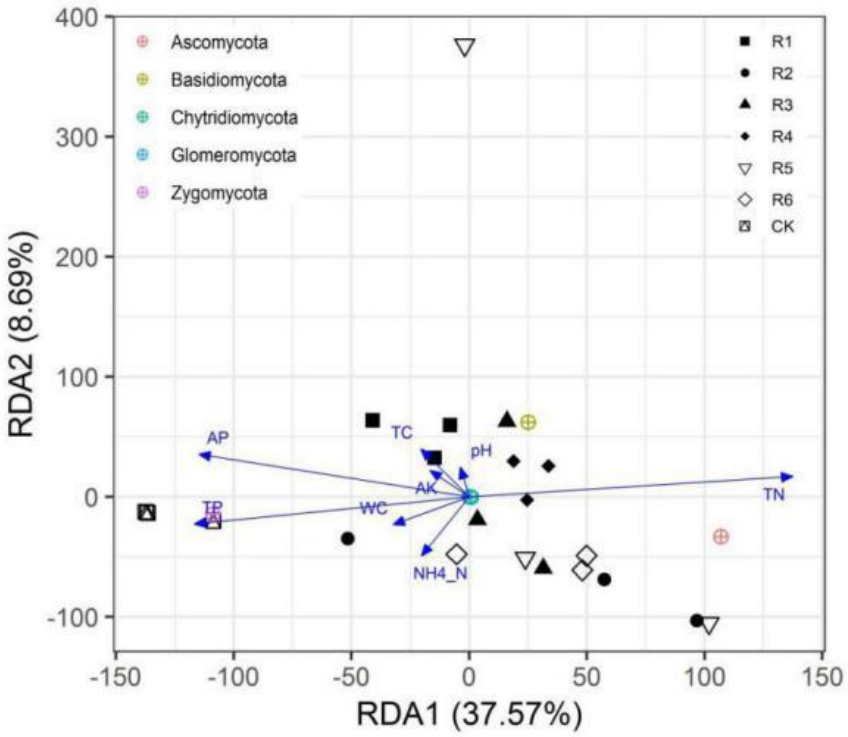
Table 4. Correlation between fungal diversity indices and physicochemical soil parameters.

Soil parameter	S_{obs} index	Shannon index	Chao1 index
WC	−0.448*	−0.549**	−0.426
pH	−0.119	0.081	−0.262
NH_4_N	−0.099	0.373	−0.227

AP	−0.219	−0.369	−0.227
AK	0.035	−0.385	0.220
TN	0.320	0.169	0.293
TC	−0.335	−0.281	−0.414
TP	−0.222	−0.323	−0.131

308 WC: soil water content; NH_4-N: ammonium nitrogen; AP: available phosphorus; AK: available potassium; TN:
309 total nitrogen; TC: total carbon; TP: total phosphorus; Spearman correlation analysis: * P <0.05; ** P <0.01

310 The results of the RDA for fungal communities and physicochemical soil factors are presented in
311 Figure 7. RDA1 and RDA2 accounted for 37.57% and 8.69% of the fungal community composition
312 in soil, respectively. Among the eight physicochemical soil factors, AP, TP, and TN affected the fungal
313 community structure most significantly, but the effects of AP and TP were the opposite of those of TN.



314

315 **Figure 7.** Redundancy analysis (RDA) for rhizosphere soil fungi and edaphic physicochemical factors
316 in artificial phytocommunities.

317 Thus, TP, AP, and TN were the primary edaphic factors that affected the fungal community
318 structure in the rhizosphere soil layer of the phytocommunities dominated by CK, R1, and R6. In
319 addition, TP had a measurable effect on the richness of *Ascomycota*, *Basidiomycota*, and

320 *Zygomycota*, whereas TN affected the richness of *Zygomycota* only (Table 5).

321 **Table 5.** Correlation between fungal species abundance indices and physicochemical soil parameters.

Soil parameter	*Ascomycota*	*Zygomycota*	*Basidiomycota*	*Unclassified_k_Fungi*
WC	−0.121	0.113	−0.416	−0.004
pH	0.040	−0.291	−0.086	0.074
NH_4_N	0.093	−0.083	0.014	0.213
AP	−0.348	0.161	−0.338	0.191
AK	−0.115	0.133	−0.120	−0.027
TN	0.291	−0.575**	0.292	−0.300
TC	−0.021	−0.218	0.002	−0.028
TP	−0.520*	0.460*	−0.510*	0.504*

322 WC: soil water content; NH_4-N: ammonium nitrogen; AP: available phosphorus; AK: available potassium; TN: total
323 nitrogen; TC: total carbon; TP: total phosphorus; Spearman correlation analysis: * P <0.05; ** P <0.01.

324 Among the edaphic factors, TP was significantly negatively correlated with the richness of
325 *Ascomycota* and *Basidiomycota* ($p < 0.05$), but significantly positively correlated with the richness of
326 *Zygomycota* ($p < 0.05$); and TN was significantly negatively correlated with the richness of
327 *Zygomycota* ($p < 0.01$).

328 ## 4. Discussion

329 More than 90% of China's coal reserves are exploited by underground mining, which is linked to
330 ground surface subsidence and other environmentally disruptive events [20]. Coal mining subsidence
331 has a severe impact on the ecosystems in mining regions, causing various problems such as changes
332 in the natural landscape, subsidence fractures, and damage to soil structure [21–23]. In addition, coal
333 mining subsidence harms the root systems of plants [24], affects their ability to absorb water and
334 nutrients [25,26], and thus, has an adverse effect on plant growth and development [27–29]. Moreover,
335 ground surface subsidence reduces soil fertility, along with the richness and diversity of soil microbes
336 [4].

337 Soil microbes are a critical factor in many ecosystem processes, and microbial diversity indicates

their functions in ecosystems [30]. To ensure the sustainability of soil ecosystems, it is of crucial importance to restore the activities and communities of soil microbes [31]. The root system activities and root exudates of transplanted trees are beneficial to the properties of degraded soil [32]. To achieve environmental remediation in abandoned mining land, appropriate measures have to be implemented, such as screening, cultivating, and transplanting of suitable pioneer tree species [33], introducing soil improvement and fertilization measures [34–35], and reclaiming the land by afforestation [2] and restoring soil microbial communities [36]. Different types of land utilization and measures of vegetation restoration can affect the physiochemical properties and microbial communities of soil [37,38].

The various plants grown in the Loess Plateau had different effects on the physiochemical properties of soil [39]. The plant diversity, quantity, and selection of the planted vegetation may affect the microbial diversity of the soil [40]. Both the quality and quantity of residuals returned to the soil vary with plant species [41]. Vegetation restoration can be achieved by adjusting the soil water content and temperature, improving the soil quality, and restoring soil ecosystems. These measures require the monitoring of soil parameters including the microbes, pH, TC, and TN [42]. Earlier reports have shown that tree species and soil fertility affect the composition and structure of rhizosphere microbial communities [43,44]. Although the planted trees and grass can cause secondary disturbance to areas affected by coal mining subsidence, related studies have shown that tree transplanting, grass seed planting, and microbial remediation [45,36] improve the vegetation coverage and increase plant adverse resistance and productivity [46-48], and promote the microbial diversity and community structure in soil [49].

Increased vegetation coverage has a positive effect on soil microbial biomass [50]. Successfully transplanted trees induce changes in the phytocommunities, soil microbial diversity, and soil properties [51–53]. Live oaks, sawtooth oaks, Mongolian oaks, *Amorpha fruticosa*, and elms can affect rhizosphere microbial diversity and plant growth [54,55]. For example, *R5*, *Prunus humilis*, *Xanthoceras sorbifolia*,R6, and *Robinia pseudoacacia* generate large quantities of photosynthetic carbon to support the growth of soil fungi and positively affect the diversity of soil fungi by the energy acquired from plant residue [56,57]. The comparison between bare soil and rhizosphere soil with plants

366 revealed that vegetation restoration can improve the physiochemical properties of soil significantly
367 [58]. For example, the microbial biomass of rhizosphere soil with transplanted trees was higher than
368 that of bare the soil and increased with the degree of vegetation restoration [59]. Other studies showed
369 that increased plant richness increased soil fungal diversity in tropical jungles [60,61] and grassland
370 [62,63].

371 The present study found that, in the areas affected by coal mining subsidence of the Loess Plateau,
372 the diversity of rhizosphere soil microbes varied with the species of transplanted trees. Specifically,
373 the R5 community had the highest S_{obs} and Chao1 indices of rhizosphere soil bacteria, the R2
374 community had the highest S_{obs} and Chao1 indices of rhizosphere soil fungi, and the R4 community
375 displayed the highest Shannon indices of rhizosphere soil bacteria and fungi. These indices were
376 higher than the corresponding indices of the primary CK phytocommunity in mining subsidence areas.
377 The transplanted R4, R5, and R2 had changed the richness and diversity of soil bacteria and fungi
378 significantly, indicating that the transplanted trees in the subsidence areas increased the diversity of
379 microbial communities in the soil. These results were similar to those of previous studies [2,64].

380 The present study also found that the soil bacterial composition of the R2 phytocommunity
381 differed from that of the primary CK phytocommunity most significantly, whereas those of the *R5* and
382 *R4* phytocommunities varied slightly from that of the primary CK phytocommunity. The soil fungal
383 composition of the R3 community differed slightly from that of the primary CK phytocommunity.
384 Moreover, the species richness of *Proteobacteria* in the rhizosphere soil of the transplanted trees was
385 higher than that of the CK phytocommunity. *Proteobacteria* was the second largest bacterial phylum,
386 which has an important role in the global cycles of C, N, and S [65]. These bacteria vary significantly
387 in form, physiology, and metabolic diversity. The species richness of *Ascomycota* and *Basidiomycota*
388 in the rhizosphere soil of various transplanted tree species was higher than that of CK in areas affected
389 by coal mining subsidence. This analysis revealed that the composition and structure of microbial
390 communities in rhizosphere soil varied significantly with the tree species [66]. The transplanted tree
391 species had significant effects on the composition and structure of microbial communities in the soil
392 of primary phytocommunities and are key factors influencing microbial diversity [43,44, 67].

393 Physicochemical soil parameters are among the major influencing factors for soil microbial

394 diversity, which they affect more significantly than aboveground vegetation [68–70]. In a forest
395 ecosystem, different tree species can affect the composition and activity of bacterial communities in
396 the soil indirectly by affecting the physiochemical properties (e.g., pH value, organic content, nitrogen
397 content, and carbon-nitrogen ratio) of the soil [67,44]. These changes in physiochemical properties
398 can partially account for the effects of tree species on soil microbes [44,43]. Some studies on
399 vegetation restoration in mining areas by tree planting have argued that plants can affect the microbial
400 properties of soil by changing the availability of soil nitrogen, and the major planted tree species can
401 significantly affect the microbial community composition at the phylum level in the soil, specifically
402 for soil bacteria (e.g., *Actinobacteria*, *Planctomycetes*, and *Nitrospirae*) and species richness at the
403 phylum level in soil fungi (e.g., *Ascomycota, Basidiomycota,* and *Zygomycota*) [44].

404 Our study found that various physicochemical soil factors had different effects on the microbial
405 diversity in areas affected by coal mining subsidence; specifically, the TN, TP, WC, and pH of the soil
406 mainly affected the S_{obs}, Shannon, and Chao1 indices of soil bacteria. The soil WC mainly affected
407 the S_{obs} and Shannon indices of soil fungi, and the soil TN mainly influenced the species richness of
408 *Actinobacteria* (a bacterium) and *Zygomycota* (a fungus). These findings implied that the changes in
409 soil microbes occurring in the phytocommunities of transplanted trees could be driven by the
410 physicochemical soil properties, e.g., the quality of soil carbon and nitrogen and the root exudates of
411 plants [53]. Soil bacteria are highly sensitive to the changes in soil nutrients [71,72], and the microbial
412 composition is significantly affected by the carbon input of root systems, the physiochemical
413 properties of soil, and the interactions between plant species [73,74].

414 Some forest types affected the fungal communities in the soil more significantly. This occurred
415 because soil fungi are more closely associated with plants (they have a symbiotic relationship via the
416 root systems) than soil bacteria [54]. This further demonstrates that the changes in aboveground
417 phytocommunities can alter the composition and structure of microbial communities in the soil [74].
418 The findings of this study support this conclusion; specifically, the observation that the different
419 transplanted tree species in the areas affected by coal mining subsidence affected the physicochemical
420 properties of soil, as well as the composition and diversity of the microbial communities in soil. These
421 changes had indirect effects on the structural characteristics of the soil bacteria by changing the

availability of soil nutrients [74,44,43].

5. Conclusions

In the areas affected by coal mining subsidence of loess regions in western China, some dominant bacterial (e.g., *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, and *Acidobacteria*) and fungal (e.g., *Ascomycota* and *Zygomycota*) phyla displayed a high species richness.

Transplanted trees altered the diversity and community structure of soil bacteria and fungi in areas affected by coal mining subsidence, and increased their species richness and diversity ($p < 0.05$). Specifically, *R4* had the highest bacterial diversity in the rhizosphere soil layer, and *R2* and *R4* had the highest rhizosphere fungi richness and diversity. The transplanted tree species increased the richness among *Proteobacteria* and *Ascomycota* in the study area.

Edaphic factors affected the diversity and community structure of soil microbes; specifically, the soil TN, TP, WC, and pH had a significant correlation with the S_{obs}, Shannon, and Chao1 indices of bacteria. AP and TN affected the bacterial community structure in the rhizosphere soil layer of the *CK* phytocommunity, along with the bacterial community structure in the phytocommunities of *R2*, *R4,* andR6. The soil parameters TN, WC, and AP mainly affected the species richness of *Actinobacteria* and *Proteobacteria*, but WC was significantly negatively correlated with the fungal richness indices (i.e., S_{obs} and Shannon). The soil parameters TP, AP, and TN mainly influenced the fungal community structure in the rhizosphere of *CK*, *R1,* andR6 phytocommunities. TP mainly affected the species richness of *Ascomycota*, *Basidiomycota,* and *Zygomycota*; and, in addition, TN affected that of *Zygomycota*.

Among the phytocommunities of transplanted trees, the community structure of the rhizosphere bacteria differed from that of the rhizosphere fungi, and their respective communities were differently affected by edaphic factors. Thus, further studies should explore the underlying mechanisms, specifically, potential correlations with seasonal variations, the severity of mining subsidence, and the timeline of the vegetation restoration efforts.

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