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Soil Characteristics and Response Mechanism of the Microbial Community in a Coal–Grain Compound Area with High Groundwater Levels

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Abstract: Coal mining has led to escalating ecological and environmental issues in significant coal and grain production areas, posing a severe danger to food security. This study examines the disturbance patterns of soil factors and microbial communities in coal and grain production areas, and attempts to understand the impact of subsidence and water accumulation stress on soil characteristics and microbial communities in coal mining subsidence areas with high subsidence levels. Five specific regions of Zhao Gu Yi Mine, situated in Henan Province and under the ownership of Jiaozuo Coal Group, were chosen. Aside from the control group (CK), the study blocks situated in the coal mining subsidence zones consisted of perennial subsidence ponding (PSP), seasonal subsidence ponding (SSP), the neutral zone (NZ), and the horizontal deformation zone (HDZ). The soil nutrient indices and the stoichiometric properties of soil C, N, and P were assessed on the surface of each block. The organization of the soil microbial community was identified using high-throughput sequencing. The findings indicate that: 1. Substantial disparities exist in soil properties and microbial community structure between the subsidence and non-subsidence zones. The levels of soil organic mater (SOM), total nitrogen (TN), total phosphorus (TP), available nitrogen (AN), and available phosphorus (AP) all decrease to different extents in the subsidence area. Additionally, the coal mining subsidence waterlogged area exhibits higher levels compared to the coal mining subsidence non-waterlogged area. Conversely, the soil water content (SWC), C/N ratio, C/P ratio, and N/P ratio all increase to varying degrees. 2. Regarding the composition of the community, the presence of Proteobacteria is considerably greater in the non-water-logged area of coal mining subsidence (NZ, HDZ) compared to the water-logged area and control group ($p < 0.05$). The prevalence of Firmicutes in the subsidence water area was substantially greater compared to both the subsidence non-waterlogged area and the control group ($p < 0.05$). The prevalence of Gemmatimonadota is markedly greater in the waterlogged area of mining subsidence compared to the non-waterlogged area and CK ($p < 0.05$). The Ascomycota population reached its highest value in the neutral zone (NZ), which was significantly greater than the values observed in the seasonal subsidence ponding (SSP) and perennial subsidence ponding (PSP) regions ($p < 0.05$). On the other hand, the Rozellomycota population had its highest value in the SSP region, which was significantly greater than the values observed in the other regions ($p < 0.05$). 3. The abundance and variety of soil bacteria and fungi, as well as their important populations, are associated with different levels of soil characteristics. The primary elements that influence the alteration of microbial communities are soil nutrients and soil water content. The presence of coal mine subsidence and water accumulation has a notable impact on the properties of the soil in the surrounding area. This study offers a scientific foundation for reclaiming land affected by subsidence caused by coal mining in regions where coal and grain production are the dominant industries.

Keywords: mining area reclamation; coal–grain composite area; coal mining subsidence; microbial community; amplicon sequencing

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1. Introduction

Coal resources have been and will continue to be the primary source of energy in China for an extended period of time. They serve a crucial role in safeguarding national security [1]. Mining activities typically result in detrimental disruptions to the surface, leading to alterations in the soil's ecological environment [2] and negative impacts on soil microbial communities [3].The coal–grain composite area in the center sections of the plain is particularly notable for its deep subterranean subsidence level, intricate geological structure, and significant overlap of grain and coal production [4]. Coal mining results in significant surface subsidence, leading to the formation of a subsidence basin that is considerably larger than the gob area. This subsidence basin is accompanied by the buildup of water [5]. As a result of the elevated water level in the central area of the basin, water will accumulate. The uneven settling of the surface will cause the inner edge of the basin to slope, while the outer edge will experience significant stretching and deformation, making it prone to surface cracks [6]. During the rainy season, stagnant water accumulates and forms a lake, disrupting the local water system and circulation. This results in the direct release of water from mining and farms, which can quickly lead to water eutrophication. Consequently, the regional ecology and food security are put at risk [7]. To mitigate the aforementioned risks, the primary approach to reclaiming the high subsidence condition in the mining area in the central and eastern plain is through the reclamation of farmlands [8]. Nevertheless, the limited soil fertility and inefficient planting methods on recovered farmland have been dampening the excitement of local reclamation efforts and farmers.

The reclaimed soil in the mining area of the central plain has low fertility, poor aggregate structure, and weak functioning [9]. This is primarily due to the seasonal variation in the water accumulation level, which causes the cultivated layer of the receiving soil to easily become alkaline, thereby impacting the development and functional reconstruction of the microbial community [5,10]. Soil microorganisms are essential for the process of soil nutrient cycling, since they have a significant impact on the turnover of soil organic matter, nutrients turnover, and the overall biogeochemical cycling [11]. Soil microorganisms have a direct impact on the presence of metal oxides in soil, facilitate the production of amino sugars, and eventually enhance the buildup of soil organic carbon [12]. The fixed nitrogen present in the environment can undergo conversion into microbial biomass nitrogen. This nitrogen can then be mineralized or transformed into stable soil organic matter, facilitating the transportation and alteration of soil nitrogen [13]. Rhizobacterium is commonly believed to exert a beneficial influence on plant growth by producing iron transporters and plant hormones that promote the mobilization of phosphorus, the fixation of atmospheric nitrogen, and the fixation of iron [14]. The diversity of microorganisms in the soil has a role in preserving the functionality of the ecosystem [15]. Soil microorganisms have a significant impact on crop development, production, and quality. They also contribute to the maintenance of soil productivity, resistance against abiotic stress, and restoration of crucial functions of the soil [16]. Simultaneously, alterations in soil microbial community composition have been observed across several land use categories [17,18]. Hence, the alteration in the composition of soil microbial communities can serve as a reliable indicator for assessing the soil quality of mine reclamation efforts [19].

The previous studies have focused on comparing the unexploited, reclamation, and subsidence areas in a comprehensive manner [20–22]. Song Ziheng conducted research on the Shangwan Coal mine in Inner Mongolia. The initial area chosen for this study was the subsidence margin area. Through iso-distance selection, it was determined that the impact of subsidence on the unexploited area was primarily concentrated proximate to the edge, specifically within a distance of less than 300 m. The variety of bacteria and fungi is highly responsive to changes in soil components, and specific populations are associated with certain soil factors to different extents [23]. Zhao Jiao et al. found that altering the method of vegetation restoration can influence the microbial community and the function of soil bacteria and fungi in a mining area [24]. Zhang Qi conducted a study on the correlation between the diversity, structure, and taxonomy of soil microbial communities in slope subsidence zones of the Daliuta Coal mine in Shanxi Province [25]. A study reveals that land surface subsidence negatively impacts the living environment of vegetation and bacterium populations, leading to the formation of new topographic features. Another study reported that the composition of bacterial communities in the rhizosphere changed depending on the level of soil disturbance and moisture, and their interactions [26]. Gao et al. recently employed 16srRNA gene sequencing technology to investigate the impact of coal mine subsidence on the distribution of microbiota in farmland and its functional genes. Gao et al. (2023) discovered the presence of core microbial communities in coal mine subsidence areas. These communities were found to alter the distribution of microbial communities and their functional genes in farmland. The researchers suggested that soil moisture, pH, NH^{4+} and Ca^{2+} concentrations were the primary factors influencing the distribution of microbial communities and their functional genes [27].

Although previous researchers have conducted extensive studies on the effects of mining subsidence on soil properties and microbial community structure, there is limited research on the combined impact of mining subsidence and waterlogging on these factors. Hence, the objective of this investigation is as follows: (1) studying the soil in the subsidence area of the Zhao Gu Yi Mine, a deep-seated mine in Jiaozuo, to learn about the distribution patterns of soil characteristics and the composition of soil microbes (2) to examine the impact of mining subsidence and water buildup on soil parameters and the structure and distribution of soil microbial communities. This research aims to offer scientific theoretical direction for the successful restoration of regional ecosystems and implementation of targeted reclamation efforts.

2. Materials and Methods

2.1. Study Area

The Zhao Gu Yi Mine field is situated in the eastern section of the Jiaozuo coal field, specifically between longitudes 113°33′00″ E and 113°43′39″ E, and latitudes 35°23′09″ N and 35°28′00″ N. The climate is characterized as a warm temperate continental climate, with an average annual temperature ranging from 14.1 to 14.9 °C and an average annual precipitation of 580 to 600 mm. The yearly evaporation ranges from 1680 to 2041 mm, with the rainy season occurring in July and August each year, contributing to approximately 70% of the total annual precipitation. Field investigation reveals the existence of waterlogging in the damaged sections with most severe subsidence value of 4.21 m in the western part of the mining area. The overall extent of damage covers 1192.19 hm^2, out of which 205.39 hm^2 have had a subsidence of more than 3000 mm and have an ongoing presence of water. The subsidence depth in the area is between 1000 and 3000 mm, covering an area of 352.13 hm^2 with a water level changing continuously.

2.2. Soil Sampling

In May 2023, a typical region affected by mining subsidence was chosen in Zhao Gu Yi Mine in Henan province for sampling. The research area was categorized into subsidence and non-subsidence areas based on the specific damage types (neutral areas and horizontal deformation areas) and land use types (perennial subsidence, seasonal subsidence, and non-subsidence areas). Resulting water bodies from mining activities were categorized into two types: perennial subsidence ponding (PSP) and seasonal subsidence ponding (SSP). The non-hydrostatic region was partitioned into a neutral zone (NZ) and a horizontal deformation zone (HDZ) based on stress level. Additionally, a control group (CK) was also established in an area unaffected by subsidence. There was a total of five sets of test plots in 5 sampling belts (Figure 1). Each sample belt contained 9 sampling points, which were arranged as repeated measurements. There was a total of 45 sampling points, each sizing 10 m × 10 m. A sterilized soil drill was used to collect a soil sample from the surface (0–20 cm) after removing humus, plant roots, pebbles, and other debris from the soil surface. The specimens were well blended and transferred into a sampling bag before being placed into a portable incubator. After collection, all samples were promptly brought back to the laboratory. The soil samples were divided into two portions, one being held in a refrigerator at a temperature of 4 °C for laboratory analysis of physical and chemical properties, and one set of fresh soil samples being refrigerated, sealed in a foam box, and sent to Major Biobio-Pharm Technology (Shanghai) for sequencing.

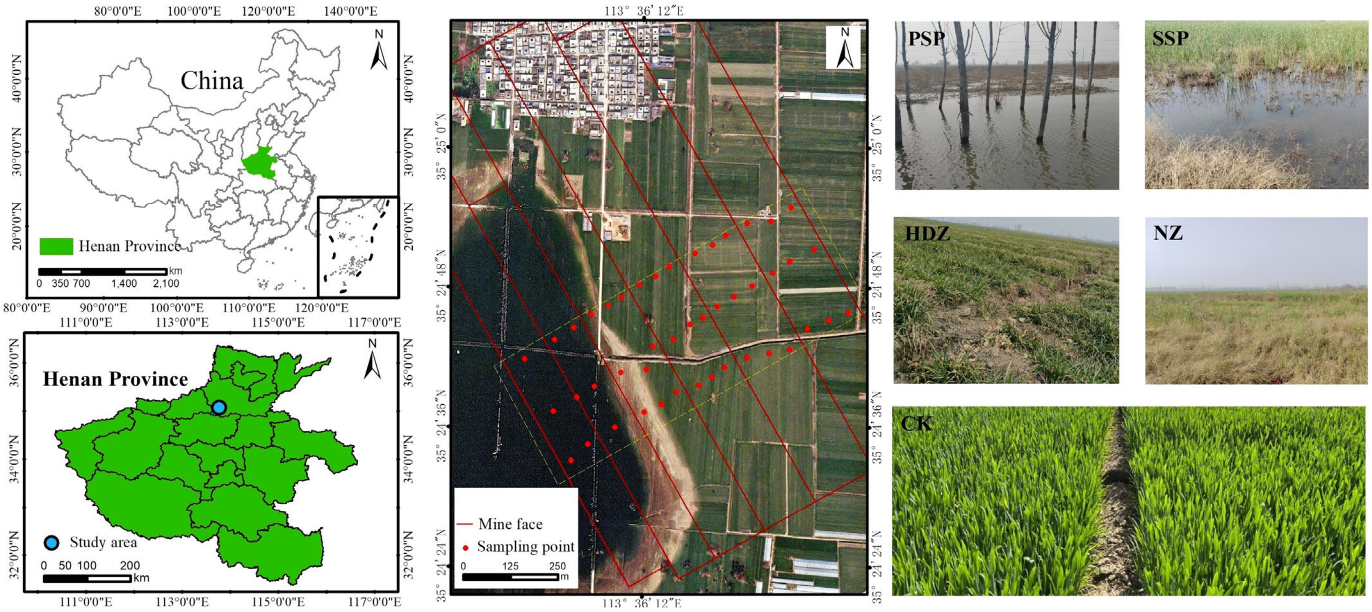

Figure 1. Geographical location and sampling points distribution over the study area. Note: PSP: perennial subsidence ponding; SSP: seasonal subsidence ponding; HDZ: horizontal deformation zone; NZ: neutral zone; CK: CalvinKlein.

2.3. Determination of Soil Characteristics

The samples that were sent to the laboratory underwent natural air drying and grinding, and were sieved through a 2 mm mesh size. After collecting, samples were then grinded and screened using mesh sizes of 0.15 and 1 mm for numbering and classification. Finally, 100 g of each sample were separated for testing.The soil pH was measured using the potentiometric method (DS-307A, Shanghai Thunder Magnetic, Shanghai, China), while the moisture content of the soil was assessed through the gravimetric drying method. Available phosphorus and total phosphorus were measured using an ultraviolet/visible spectrophotometer. Potassium dichromate and external heating were used to oxidize soil organic carbon. The semi-micro-Kelvin technique was used to calculate total nitrogen.

2.4. DNA Extraction and PCR Amplification

The E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) was used to extract the whole genomic DNA of the microbiological samples (**** samples), following the instructions provided by the manufacturer. The DNA's purity and concentration were assessed using 1.0% agarose gel electrophoresis and a NanoDrop® ND-2000 spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA). The DNA was then stored at −80 °C until it was needed for subsequent experiments. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified using the primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) using an ABI GeneAmp® 9700 PCR thermocycler (ABI, Tampa, CA, USA). The PCR reaction mixture consists of 4 μL of 5 × Fast Pfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of Fast Pfu polymerase, 10 ng of template DNA, and ddH2O added to reach a final volume of 20 μL. The PCR amplification cycling conditions consisted of an initial denaturation step at 95 °C for 3 min, followed by 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s. The last step involved a single extension at 72 °C for 10 min, followed by cooling to 4 °C. The amplification of all samples was performed in triplicate. The PCR product was isolated from a 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) following the instructions provided by the manufacturer. The quantity of the purified product was determined using the Quantus™ Fluorometer (Promega, Madison, WI, USA).

2.5. Statistical Analysis and Processing

Groups mean comparison was performed using the one-way analysis of variance (ANOVA) and the Tukey method (The groups mean comparison confirmed that all values passed the normality test ($p > 0.05$), indicating that they fit the criteria for traditional descriptive statistical analysis). The data analysis on the biological cloud platform (https://cloud.majorbio.com, accessed on 12 May 2023) encompasses the following: (1) Utilizing the mothur system (http://www.mothur.org/wiki/Calculators, accessed on 13 May 2023) to compute the chao1 alpha diversity index, Shannon index, and other relevant metrics. Additionally, employ the Wilcoxon rank and inspection method to analyze the variations in alpha diversity between groups. (2) We selected Nonmetric Multidimensional Scalin (NMDS) as the method for analyzing microbial β diversity. (Applying Nonmetric Multidimensional Scaling using Bray–Curtis distance, to assess the similarity of microbial community structures between samples.) Furthermore, the PERMANOVA non-parametric test was employed to examine whether there were noteworthy disparities in the microbial community composition among the various sample groups. The community Bar map was generated using the R programming language, based on the data table in tax_summary_a. The taxonomy analysis results provide information on the community structure and composition of various groups at the Phylum level. To determine if there are significant differences between these groups, a one-way ANOVA test was conducted. A hypothesis test was performed to assess the significance of differences in species abundance between distinct groups of microbial communities. The aim was to identify species that exhibited significant differences between the groups or samples. This study utilized distance-based redundancy analysis (db-RDA) to examine the impact of soil physicochemical characteristics on the soil microbial community. Correlations were computed using SPSS 22.0 to assess the impact of key soil physicochemical variables on the microbiological alpha diversity index.

3. Results

3.1. Temporal and Spatial Changes in Ecosystem Services

Table 1 shows that soil organic matter, total nitrogen, and total phosphorus have dramatically decreased in the mining subsidence region compared to the CK area, except in the perennial waterlogging area (Table 1). The difference in soil organic matter content (SOM) between the coal mining subsidence area and the control area ranged from 16.20 to 21.36 g kg^{-1}. The soil organic matter content exhibited significant fluctuation in the coal mining subsidence area, with the highest value observed for PSP and the lowest value for HDZ. An analysis of variance revealed a considerable variation in soil organic matter (SOM) among different locations ($p < 0.05$). The research area exhibited a range of total phosphorus (TP) concentrations, with values ranging from 0.96 to 1.18 g kg^{-1}. The highest concentration was observed in CK, while the lowest concentration was found in HDZ. Simultaneously, there was a significant difference in the total phosphorus levels among the locations ($p < 0.05$). The variations in total nitrogen (TN) content and soil organic matter (SOM)were comparable, although PSP exhibited a much greater value compared to HDZ, NZ, and SSP. In contrast to other nutrients, the range of variation for alkali-hydrolyzed nitrogen (AN) was 69.444–102.899 mg kg^{-1}. The concentration of AN in PSP was considerably lower than in HDZ, NZ, and SSP regions, and the differences were statistically significant ($p < 0.05$). The available phosphorus (AP) content in the mining subsidence area was significantly lower than that in the CK area, with the lowest value seen in the SSP area. The difference between these locations was statistically significant ($p < 0.05$). The pH of the coal mine subsidence area was significantly lower than that of the

CK area ($p < 0.05$), and the pH of the coal mining subsidence area ranged from 7.721 to 7.773, indicating a somewhat alkaline condition. The soil water content (SWC) ranged from 9.8% to 18.9%. The highest SWC value was seen in the PSP area, which was considerably higher in the subsidence area compared to the non-subsidence and CK areas.

Table 1. Mean comparison of soil properties in coal mining subsidence areas.

Sampling Area	**TN (g kg^{-1})**	**TP (g kg^{-1})**	**SOM (g kg^{-1})**	**AN (mg kg^{-1})**	**AP (mg kg^{-1})**
CK	1.368 ± 0.222 a	1.473 ± 0.548 a	19.6 ± 4.114 ab	102.889 ± 21.991 a	42.667 ± 31.312 a
PWSA	1.319 ± 0.136 ab	1.12 ± 0.114 b	21.356 ± 1.994 a	69.444 ± 14.8 b	19.511 ± 5.212 bc
SSWA	1.143 ± 0.242 b	0.992 ± 0.167 b	17.778 ± 1.884 b	82.333 ± 21.407 ab	14.978 ± 8.579 c
NA	0.976 ± 0.202 b	1.002 ± 0.096 b	17.178 ± 2.786 b	86.444 ± 27.668 ab	16.433 ± 4.955 bc
TCA	0.967 ± 0.204 b	1.026 ± 0.242 b	16.2 ± 2.147 b	92 ± 25.51 ab	22.589 ± 9.916 b
F	2.745	4.731	7.645	5.161	7.549
p	<0.001	<0.01	<0.001	<0.01	<0.001
Sampling area	pH	SWC (%)	C/N	C/P	N/P
CK	7.773 ± 0.27 a	14.8 ± 18.3 ab	14.255 ± 1.349 b	14.133 ± 3.763 b	0.986 ± 0.213 a
PWSA	7.727 ± 0.068 ab	18.9 ± 1.6 a	16.234 ± 1.143 ab	19.304 ± 3.091 a	1.19 ± 0.183 a
SSWA	7.721 ± 0.043 ab	18 ± 0.8 a	15.992 ± 2.859 ab	18.194 ± 2.317 a	1.153 ± 0.153 a
NA	7.59 ± 0.16 b	12.5 ± 5.2 b	18.328 ± 5.306 a	17.115 ± 2.153 a	0.99 ± 0.26 a
TCA	7.58 ± 0.111 b	9.8 ± 2.8 b	17.293 ± 3.924 ab	16.768 ± 2.528 ab	1.023 ± 0.304 a
F	4.198	1.593	2.94	1.749	1.908
p	<0.01	0.195	<0.01	0.158	0.128

Note: TN (total nitrogen), TP (total phosphorus), SOM (soil organic matter), AN (alkali-hydrolyzale nitrogen), AP (available phosphorus), and SWC (soil water content). Distinct lowercase letters following the same column of data show that the difference between the same index in different locations is statistically significant at a significance level of 0.05. F represents the statistic used in the analysis of variance, while p represents the corresponding significance level.

When comparing CK, C/N, C/P, and N/P in various regions of coal mining subsidence areas, they exhibit varying degrees of increase. Specifically, C/N was higher in water-logged areas of coal mining subsidence compared to non-water-logged areas, while C/P and N/P were also higher in water-logged areas compared to non-water-logged areas. The carbon-to-nitrogen ratio (C/N) throughout the entire research area varied between 14.255 and 18.328. The C/N ratio in the NZ region was substantially greater than in the CK region ($p < 0.05$), but no significant difference was seen in the other regions. The C/P values varied significantly among different regions, ranging from 14.133 to 19.304. The lowest value was observed in CK, which was significantly lower than in the other regions ($p < 0.05$). The research area exhibited a range of N/P values, ranging from 0.986 to 1.19. There were no significant differences seen among different regions.

3.2. Differences in Microbial Species Diversity

We evaluated the variety of soil microorganisms in various places. Figure 2 demonstrates that the chao index of bacteria ranges from 3004 to 3738 across the entire study area. There was a substantial variation in the chao index, with a noticeable decrease as the distance from the water center increases. Additionally, the chao index in different locations of the coal mining subsidence area was lower than the control group (CK). The bacteria in the CK region exhibited the highest chao index, which was substantially greater than that of the SSP region ($p < 0.001$). The Shannon index varied between 7.206 and 7.427, and exhibited a decline from the outer regions to the vicinity of the water center. The Shannon index of coal mining subsidence areas in different regions was found to be lower than that of CK. Furthermore, the Shannon index of PSP and SSP regions was significantly lower than that of CK ($p < 0.05$). The Simpson index ranged from 0.0011 to 0.0018. The Simpson index exhibited a greater value in the coal mining subsidence area compared to the CK area. Additionally, there was an increasing tendency in the distance from the water center as it moved from farther to closer regions. The Simpson index of PSP was substantially greater than that in the CK area ($p < 0.05$).

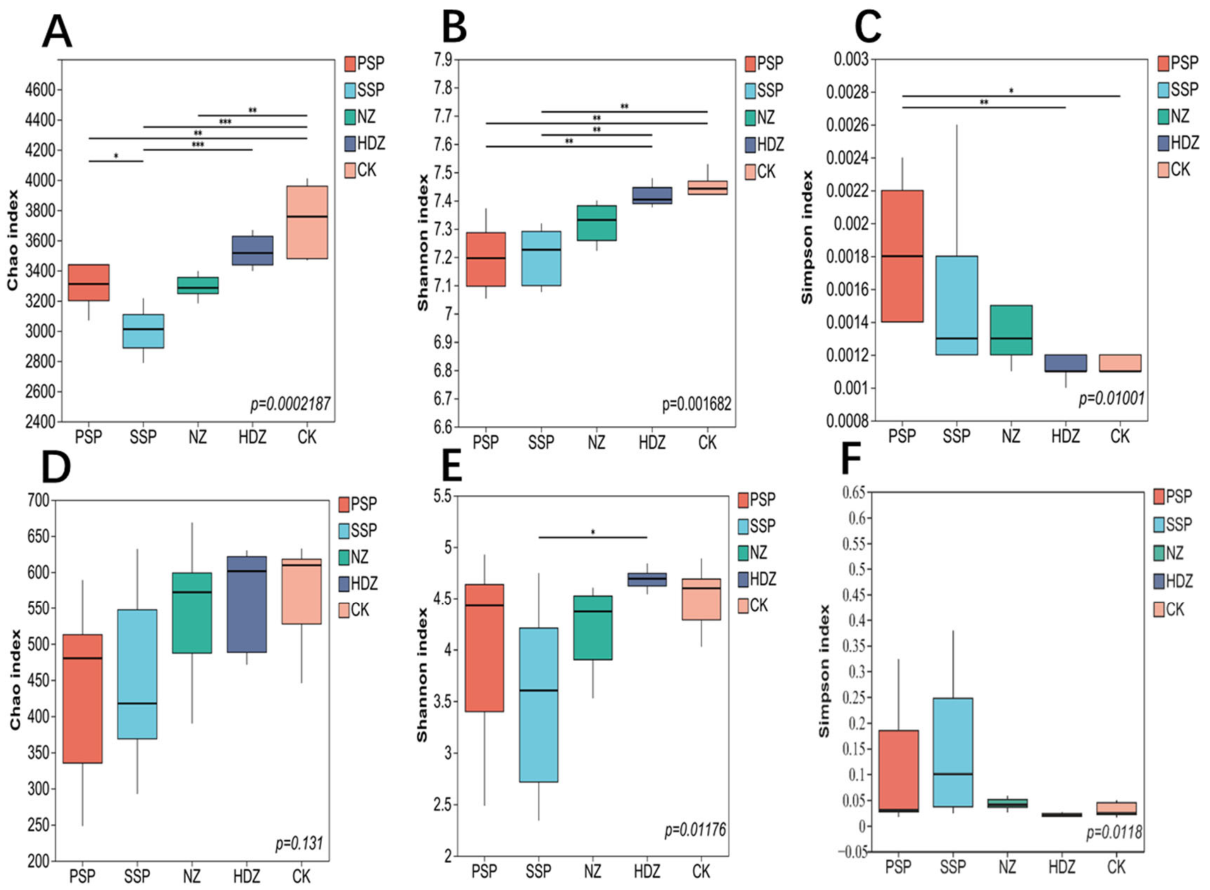

Figure 2. Diversity of bacteria (**A**–**C**) and fungi (**D**–**F**) α diversity index in different locations of coal. Note: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

As for the fungi, the chao index in the entire study region varied between 430.3 and 568.3, whereas the Simpson index ranged from 0.1531 to 0.0211. The chao index and Simpson index showed no discernible disparity between the control area and the coal mining subsidence area. The Shannon index varied between 3.505 and 4.686. The highest value was observed in the HDZ zone and was substantially greater than that in the SSP region ($p < 0.05$).

Nonmetric Multidimensional Scaling (NMDS) is a technique used to reduce the complexity of a multidimensional space in order to locate, analyze, classify, and preserve the original relationships between objects(Figure 3). The primary focus is on the variation in the structure of microbial communities across several sample locations. The stress coefficient, with a value of less than 0.2, accurately reflects the test results of bacteria and fungi. Furthermore, the beta diversity of soil bacteria and fungi varies dramatically across different locations. There were notable variations in the beta diversity of soil bacteria and fungus between the water-logged area affected by coal mining subsidence and the control group (CK).

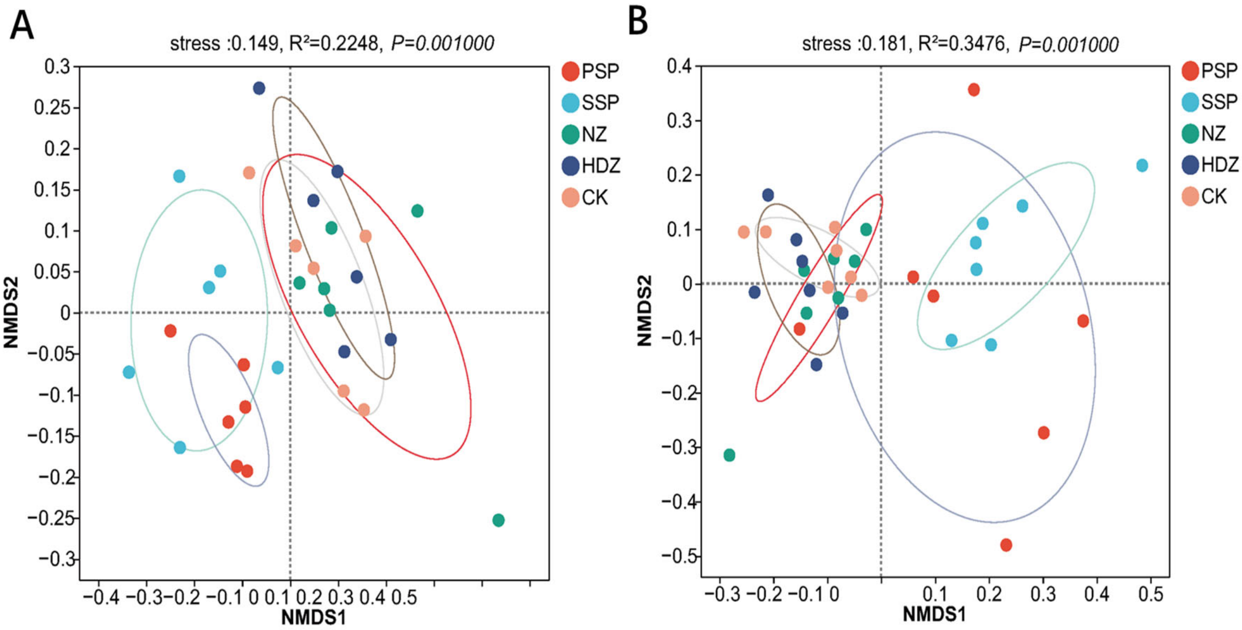

Figure 3. Non-metric Multidimensional Scaling (NMDS) analysis based on the Bray-Curtis distance of bacteria (**A**) and fungi (**B**). The ellipses in the figure illustrate grouped ellipses formed between sample points in different study areas;PSP: perennial subsidence ponding; SSP: seasonal subsidence ponding; HDZ: horizontal defor-mation zone; NZ: neutral zone; CK: CalvinKlein.

3.3. Differences in Soil Microbial Community Composition

For bacteria, an analysis was conducted on the diversity data of 45 samples, resulting in a total of 3,264,926, 1,369,322,862 bases. The average sequence length was found to be 419bp. The clustering process resulted in a total of 87,918 ASVs. Figure 4A clearly demonstrates a significant disparity in the composition of bacterial populations ($p < 0.05$). The taxonomic analysis revealed the species and relative abundance of key microbial phyla in the samples collected from the coal mining subsidence region and the control area of Zhao Gu Yi Mine. The analysis was conducted at the phylum level, and the findings are presented below. The predominant bacterial groups in the coal mining subsidence and the control area consist of Proteobacteria, Acidobacteriota, Actinobacteriota, Firmicutes, Bacteroidota, Chloroflexi, Myxococcota, and Gemmati, respectively, monadota. These eight phyla collectively make up over 90% of the total phyla, indicating their dominance in the ecosystem. The comparison study reveals that the primary microphyla in the subsidence area resulting from coal mining disturbance in Zhao Gu Yi Mine are essentially comparable to those in the control area, and there has been no major alteration in the sequence of relative abundance. Upon comparing the relative abundance of each phylum in different groups (Figure 4C), it was observed that the abundance of Proteobacteria in the non-water-logged area of coal mining subsidence (NZ, HDZ) was significantly greater than that in the water-logged area and the control group ($p < 0.05$). The prevalence of Firmicutes in the subsidence water area was markedly greater than in the non-subsidence water area and the control group (CK). Gemmatimonadota is more abundant in mining subsidence wet areas compared to non-waterlogged areas and CK ($p < 0.05$).

For fungi, we analyzed the diversity data of 45 samples. A total of 3,543,132,859,034,147 bases were optimized, with an average sequence length of 243 bp. Through clustering, a total of 8150 Amplicon Sequence Variants (ASVs) were obtained. The composition of bacterial communities exhibited a significant disparity, as depicted in Figure 4B. The taxonomic analysis revealed the species and relative abundance of the major microbial phyla in samples collected from the coal mining subsidence region and the control area of Zhao Gu Yi Mine. The analysis was conducted at the phylum level, and the findings are presented below. The predominant bacterial phyla found in the coal mine subsidence area and the control area included Ascomycota, Mortierellomycota, Rozellomycota, Basidiomycota, and others. The combined prevalence of these five phyla constituted over 90% of the overall phylum, representing the prevailing phyla. Comparative research revealed notable disparities among several localities (Figure 4D). The Ascomycota group had its highest value in the NZ region, which was significantly greater than the values observed in the SSP and PSP regions ($p < 0.05$). On the other hand, the Rozellomycota group had its highest value in the SSP region, which was significantly higher than the values observed in the other regions ($p < 0.05$). The findings indicated that the subsidence of coal mining at Zhao Gu Yi Mine had a discernible impact on the composition of the soil microbial community. The subsidence of coal mining has resulted in alterations to the organization of soil microbial communities, as they have adapted to the new environmental conditions.

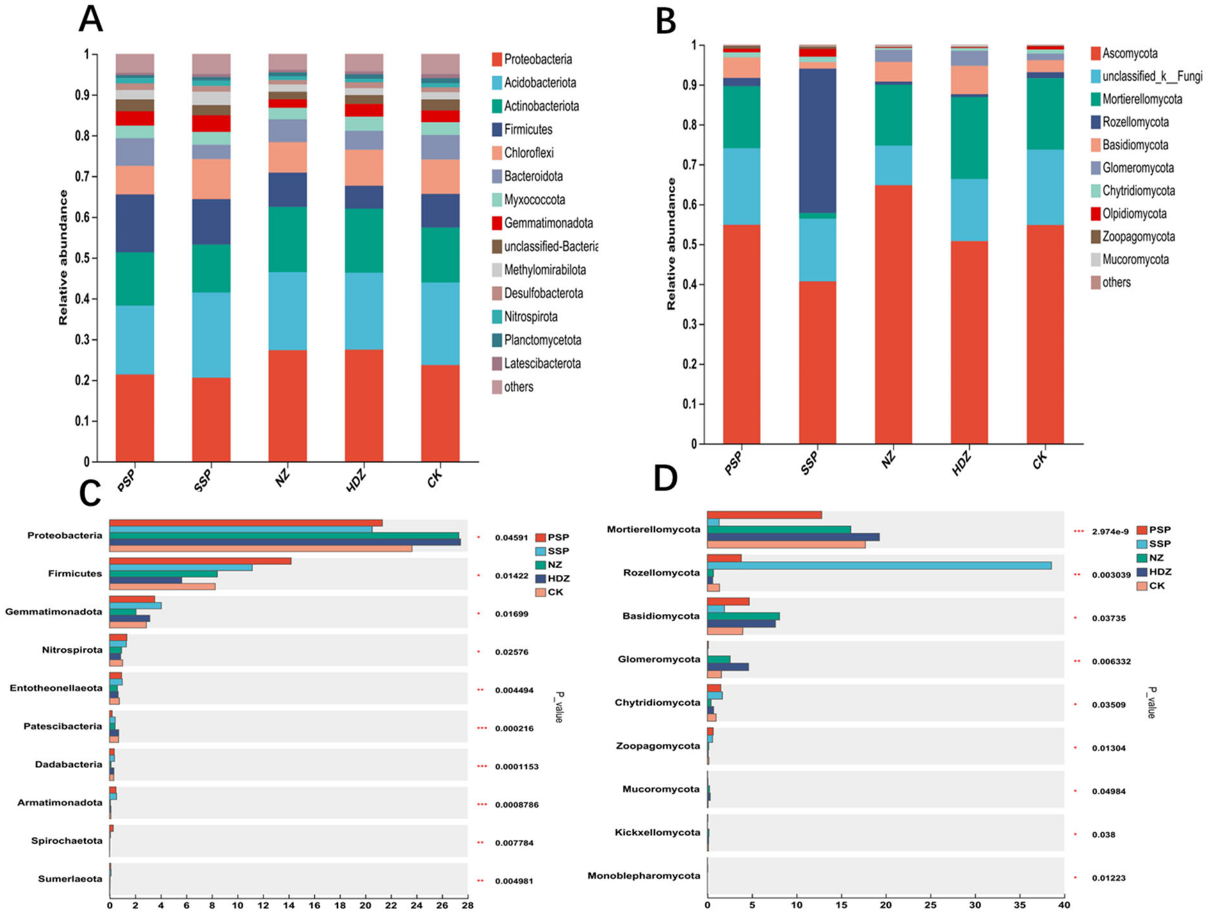

Figure 4. Relative abundance of the top 15 of soil bacterial (**A**) and fungal (**B**) communities at the phylum level. Relative abundance bacterial (**C**) and fungal (**D**) phylum that showed significant differences in different locations of coal. The Kruskal–Wallis H test was used to evaluate the significance of differences between the indicated groups. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3.4. The Relationship between Soil Microbial Community and Environmental Factors

Pearson's correlation analysis (Table 2) revealed strong positive correlations between the chao index and pH, AP, and TP ($p < 0.01$), as well as negative correlations with N/P, C/P, and Soil water content (SWC) ($p < 0.01$). The Shannon index exhibited a positive correlation with AP and AN ($p < 0.05$), and a negative correlation with N/P, C/P, and SWC ($p < 0.01$). The Simpson index exhibited a positive correlation with moisture and SOM ($p < 0.01$), and a negative correlation with AN, AP, and N/P ($p < 0.01$). The chao index for fungi (Table 3) exhibited a negative correlation with both the C/N ratio and SWC ($p < 0.01$). The Shannon index exhibited a significant negative correlation with both SOM and SWC ($p < 0.01$). The Simpson index exhibited a positive correlation with pH and SWC

($p < 0.01$). The results demonstrated that water exerted a substantial suppressive impact on the abundance and variety of soil microorganisms.

Table 2. Correlation analysis of α diversity index of soil bacteria and soil physical properties.

Diversity Index	**TN (g kg^{-1})**	**TP (g kg^{-1})**	**SOM (g kg^{-1})**	**AN (g kg^{-1})**	**AP (g kg^{-1})**
Chao	0.011 (0.953)	0.422 (0.020 **)	−0.014 (0.940)	0.227 (0.228)	0.591 (0.001 ***)
Shannon	−0.146 (0.441)	0.199 (0.293)	−0.298 (0.110)	0.333 (0.072 *)	0.463 (0.010 **)
Simpson	0.111 (0.560)	−0.044 (0.816)	0.347 (0.060 *)	−0.364 (0.048 **)	−0.393 (0.032 **)
diversity index	pH	SWC (%)	C/N	C/P	N/P
Chao	0.366 (0.046 **)	−0.597 (0.000 ***)	−0.152 (0.421)	−0.527 (0.003 ***)	−0.507 (0.004 ***)
Shannon	0.221 (0.240)	−0.62 (0.000 ***)	−0.337 (0.068 *)	−0.463 (0.010 ***)	−0.355 (0.054 *)
Simpson	−0.009 (0.962)	0.497 (0.005 ***)	0.406 (0.026 **)	0.281 (0.133)	0.092 (0.628)

Note: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Table 3. Correlation analysis of α diversity index of soil fungi and soil physical properties.

Diversity Index	**TN (g kg^{-1})**	**TP (g kg^{-1})**	**SOM (g kg^{-1})**	**AN (g kg^{-1})**	**AP (g kg^{-1})**
Chao	0.129 (0.399)	−0.148 (0.333)	−0.124 (0.416)	0.068 (0.656)	−0.044 (0.776)
Shannon	−0.034 (0.826)	−0.108 (0.481)	−0.258 (0.087 *)	0.151 (0.323)	0.005 (0.973)
Simpson	0.075 (0.623)	0.046 (0.765)	0.248 (0.100)	−0.201 (0.185)	−0.103 (0.501)
diversity index	PH	WATER	C/N	C/P	N/P
Chao	−0.154 (0.311)	−0.331 (0.026 **)	−0.261 (0.083 *)	−0.039 (0.797)	0.167 (0.273)
Shannon	−0.221 (0.144)	−0.323 (0.031 **)	−0.191 (0.208)	−0.095 (0.535)	0.108 (0.482)
Simpson	0.337 (0.024 **)	0.414 (0.005 ***)	0.134 (0.380)	0.134 (0.379)	−0.039 (0.802)

Note: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Figure 5A demonstrates a noteworthy negative correlation ($p < 0.05$) between Proteobacteria and TN and C/P. There is a strong positive correlation between Acidobacteriota and N/P. The presence of Actinobacteriota showed a strong negative correlation with TN and N/P ratio, with a statistical significance of $p < 0.01$. The abundance of Firmicutes and Water showed a significant positive correlation ($p < 0.05$). Similarly, there was a positive correlation between Gemmatimonadota and SWC. Additionally, an unclassified_k__norank_d__Bacteria showed a positive correlation with SOM, TN, pH, and water. Regarding fungi, Figure 5B demonstrates a substantial negative correlation ($p < 0.05$) between Ascomycota and the ratios of C/P and N/P. The presence of Mortierellomycota showed a strong negative correlation with SOM, TN, C/P, N/P, and SWC ($p < 0.05$). The presence of Rozellomycota showed a significant positive relationship with SOM and SWC ($p < 0.05$). Basidiomycota showed a strong negative correlation with SOM, C/N, and SWC.

This study utilized db-RDA analysis, which is based on the distance from bray cutis, to examine the correlation between soil bacterial communities and fungi. This analysis focused on the relative abundance of the top 10 dominating phyla of bacteria and the soil physicochemical properties (Figure 6). This study area's soil bacterial community composition was significantly affected by TN, TP, AN, AP, pH, C/P, C/N, and water ($p < 0.01$). Our results revealed that TN, TP, AN, AP, pH, C/P, C/N, and SWC had a substantial impact on the overall composition of the soil bacterial population ($p < 0.01$). When considering the environmental factors mentioned above, the sample points were divided into two clusters. In NZ and HDZ, C/P and C/N had a greater impact on the composition of the bacterial community. On the other hand, in SSP, TN, TP, AN, AP, pH, and water had a greater influence on the bacterial community composition (Figure 6A). The replacement results indicated that the soil total phosphorus ($R^2 = 0.45$, $p = 0.001$) was the primary factor driving variations in different areas of the coal mining subsidence area. The variables TN, TP, SOM, C/P, and C/N had a significant impact on the overall composition of the soil fungus community ($p < 0.01$). Given the conditions described above, the sample points were grouped into two distinct clusters. The variables TN, TP, and SOM had a greater impact on the composition of the fungal community in the settled hydrops (PSP and SSP)(Figure 6B). On the other hand, the variables C/P and C/N had a greater impact on the composition of the fungal community in the non-water-logged area of coal mining subsidence (NZ and HDZ). The replacement results indicated that soil organic carbon (R^2 = 0.48, p = 0.001) was the primary factor driving variations in different areas of the coal mining subsidence area.

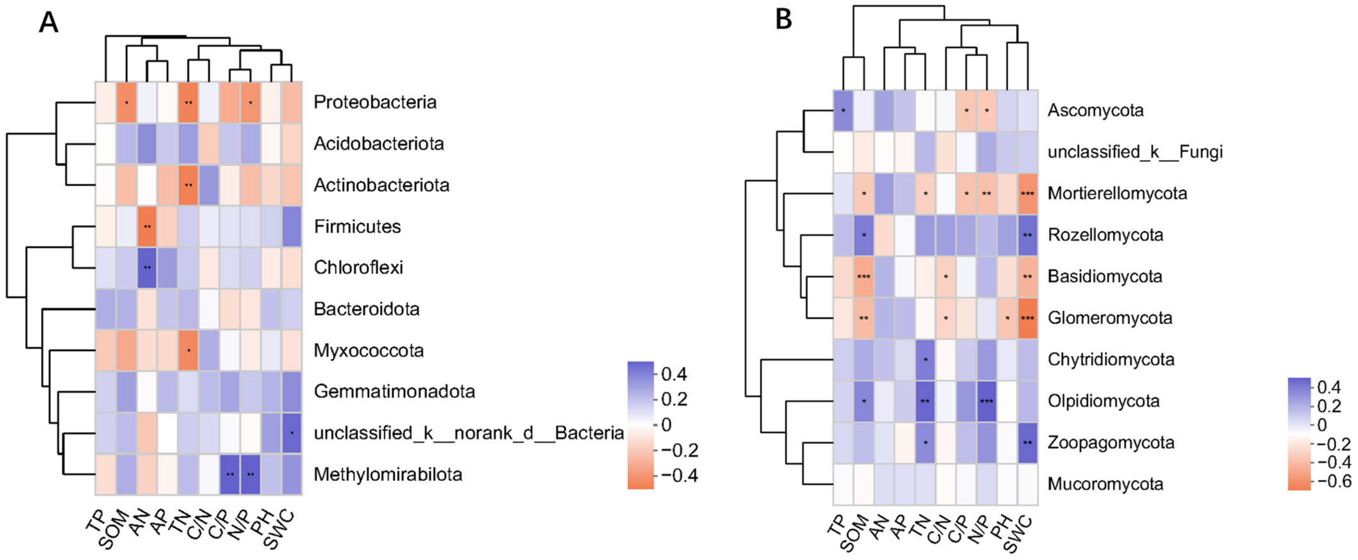

Figure 5. Heat map of correlation between of the top 10 bacterial (**A**) and fungal (**B**) phylum of soil microorganisms and soil characteristics. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

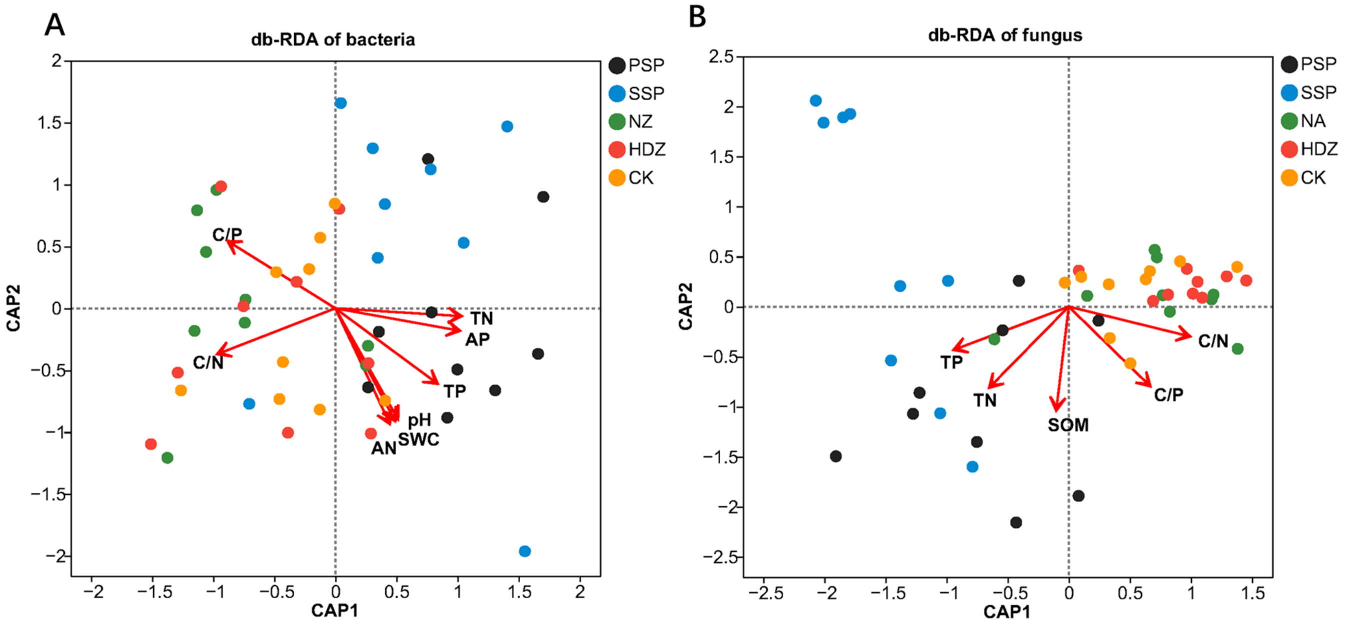

Figure 6. db-RDA analysis based on the Bray–Curtis distance of bacteria (**A**) and fungi (**B**).

4. Discussion

4.1. Subsidence and Water Accumulation Impact on Soil Properties and Microbial Community Composition

Several studies have indicated that land use can directly alter soil physiochemical properties and microbial composition. This phenomenon plays a significant role in shaping the changes observed in soil diversity [17,28,29]. The fluctuations between arid and wet conditions, as well as between aerobic and anaerobic conditions, partially contribute to the enhancement of microbial diversity [30]. This study reveals that the coal mining subsidence water area exhibits distinct wetland water conditions compared to agricultural land (CK). It also shows a transformation in the composition of the aerobic and anaerobic layers, which is expected to enhance the diversity of the soil microbial community [31]. However, the difference in diversity between the two areas was not significant. The diversity of the soil microbial community in the SSP treatment was substantially lower than that in the CK ($p < 0.05$). Coal mining disturbance in the subsidence zone causes surface deformation and the formation of a subsidence wetland. This wetland condition is highly unstable and hinders the maintenance of a diverse soil microbial community [8]. This phenomenon is especially evident in areas that experience seasonal waterlogging. In these areas, the Soil Surface Ponding (SSP) occurs at the interface between land and water in areas prone to subsidence. Over time, the SSP undergoes cycles of drying and wetting, which leads to significant salinization and soil erosion. Consequently, this process results in a decrease in the diversity of microbial communities and a decline in soil nutrient content. According to [32], coal mining disturbance significantly affects soil properties and the organization of soil microbial communities.

4.2. Soil Properties Impact on Microbial Communities Composition in Subsidence Areas

Several studies have indicated that alterations in soil physical and chemical properties have a significant role in influencing soil microorganisms in the context of coal mining disturbance. Among these alterations, soil nutrients are identified as the primary drivers of changes in microbial communities [33]. Soil microbial activity is influenced by several soil environmental conditions, and microorganisms have a vital function in the recycling of nutrients and the maintenance of soil fertility [34]. The elements C and N are the primary constraints on nutrient cycling, as stated by [35]. As mining operations continue for a longer period, subsidence will disrupt the surface, leading to varying degrees of damage and impact on surface ecology. The carbon-to-nitrogen ratio (C/N) in areas affected by coal mining subsidence will exhibit varying degrees of increase, suggesting a decline in the efficiency of soil microbial decomposition and nutrient utilization [36]. Based on db-RDA study, the causes influencing the water-logged and non-water-logged areas of coal mining subsidence varied. However, they were primarily associated with soil nutrients, with total phosphorus having the most significant impact on community distribution. Bacteria mostly consist of phosphorus, and their cell walls include a significant quantity of teichoic acid, which is essential for the growth of soil bacteria. Hence, the bacterial community is greatly influenced by the overall concentration of phosphorus [37]. The presence of coal mining disturbance and subsidence in various places leads to varying degrees of reduction in TP, hence constraining bacterial development. This study revealed that there was a notable decline in soil microbial diversity specifically in the middle section of the collapse slope (NZ and SSP). This decline was attributed to the loss of soil nutrients in the subsidence area, resulting in a decrease in microbial carbon source. Additionally, it caused alterations in the original microbial community structure, hindered microbial growth and reproduction, and led to a decrease in the relative abundance of bacteria and fungi [38]. The central section of the slope experiences more severe soil and water erosion due to its significant incline. Additionally, this area exhibits the greatest and smallest reduction in nutrient content compared to other locations on the slope. The findings of [39] indicated that the levels of soil total nitrogen and total phosphorus decreased from PSP to SSP, and the soil fertility in areas with seasonal waterlogging was lower compared to non-waterlogged areas [40], which aligns with the results of this study.

Coal mining subsidence results in landscape damage that alters the soil characteristics. Furthermore, the influence on various sites within the subsidence area varies. The study findings revealed varying degrees of soil nutrient depletion in different locations of the coal mining subsidence area, suggesting that coal mining disturbance significantly influenced soil properties. However, there was a steady increase in soil water content. Simultaneously, there exists a varying degree of relationship between the diversity of soil bacteria and fungus, as well as their important populations, with soil properties. Among these parameters, water content stands out as a primary driver of microbial community change. Soil moisture content is a significant determinant of plant growth and has a direct impact on crop yield [41]. This study found a substantial negative correlation between water content and soil microbial diversity. This is because an increase in surface soil water content leads to reduced soil aeration, resulting in sticky soil texture and ultimately inhibiting microbial and enzyme activity [42].

4.3. Soil Properties Impact on Microbial Dominant Population in Subsidence Areas

Soil microbial reclamation is a crucial method for enhancing soil quality and facilitating the restoration of biodiversity in places that have been disrupted by coal mining activities [43]. Hence, the correlation between significant functional groups of soil microorganisms and soil variables is vital for the rehabilitation of mine ecology [44]. Currently, the process of how soil bacterial communities is formed and sustained is not completely comprehended. However, by examining the variations in the components of soil bacterial communities, we can gain insight into how environmental changes affect the reconstruction of these communities in their natural habitat. The reconstruction of soil microbial community composition is determined by environmental factors, rather than the composition of transplanted soil microorganisms [45]. Although there are notable variations in soil bacterial diversity resulting from alterations in land use, these variations originated from the same communities. The soil bacterial community structure in wetlands is significantly influenced by various land use patterns [46]. According to [47], Proteobacteria, actinomycetes, and Acidobacteria are the most prevalent bacterial groups in the soil of coal mining subsidence zones. Studies have shown that some kinds of bacteria, specifically Acidobacteria, Firmicutes, and Proteobacteria, have a significant role in connecting soil bacterial communities [48]. These findings match the results of our research. The primary bacteria found in coal mine subsidence at high subsidence levels include Proteobacteria, Acidobacteriota, Actinobacteriota, and Firmicutes. The prevalence of Proteobacteria in the dry coal mining subsidence area is considerably greater than that in the coal mining subsidence area. This suggests that soil bacteria belonging to the Proteobacteria group have a preference for the dry environment of the coal mining subsidence area [49]. Proteobacteria exhibits a wide-ranging distribution and is abundant in aerobic bacteria that break down different contaminants, as well as bacteria that facilitate oxidase growth [50].The disruption caused by coal mining has led to an increase in soil porosity and a decrease in the water holding capacity of areas affected by coal mining subsidence. This has created favorable conditions for the growth of aerobic bacteria, which have become the dominating species in these places. The abundance of Firmicutes in the PSP region is substantially greater than in non-water-logged locations. Firmicutes are a group of bacteria that have a high growth rate and may rapidly proliferate in a carbon-rich environment [51,52]. A substantial quantity of organic matter collected in PSP, creates an optimal growing environment for the proliferation of Firmicutes.

Regarding fungus, ascomycetes and basidiomycetes were the predominant groups in soil fungi. The variation in soil types is responsible for the diverse soil elements that influence the development of fungal communities. Changes in soil nutrients can result in variations in soil fungal communities, leading to significant alterations in resource availability across different soil types. This, in turn, enhances the rate of microbial succession [53]. Among the various fungal groups, Ascomycetes exhibit the highest abundance and display a significant negative correlation with C/P and N/P ratios. Ascomycetes are highly numerous fungi in nature, exhibiting a faster evolutionary rate and more species diversity compared to basidiomycetes [54]. However, the precise biological function of the majority of ascomycetes remains uncertain. Therefore, it is imperative to do further research to investigate their significance in land reclamation and ecological restoration. The predominant bacteria Mortierellomycota exhibited a substantial negative correlation with soil organic matter, total nitrogen, carbon to phosphorus ratio, nitrogen to phosphorus ratio, and water content ($p < 0.05$). On the other hand, Rozellomycota showed a significant positive correlation with SOM and water content ($p < 0.05$). Basidiomycota had a significant negative correlation with SOM, carbon to nitrogen ratio, and water content. Alterations in soil nutrient levels give rise to modifications in the soil fungus community, yielding outcomes that align with the findings of previous researchers [55].

4.4. Inspiration for Precise Reclamation of Mineral-Grain Composite Area

In the high-water-level mineral-grain complex region, the surface subsidence brought about by underground coal mining can undoubtedly prompt the developed land over the minedout region to transform into wetland, the consequences of this paper show that coal mining truly influences the spatial dissemination of soil properties and microbial local area structure in coal mining subsidence region, crevices and soil disintegration in coal-mining regions lead to bring down soil supplement levels and lower security of microbial local area structure in non-waterlogged regions, notwithstanding the conveyance of soil supplements in waterlogged regions is not the same as that in non-waterlogged regions because of the increment of slant and overlying oceanic plant. Accordingly, it is the way to change the dirt into the powerful arable land in the coal–grain complex region.

Pertinent examination shows that the central issue of land recovery innovation in mining region is soil reproduction [56], developing a sensible and stable soil condition is the fundamental work of land recovery, particularly in the mineral-grain compound region, the burn characteristics of remade soil layers ought to be profoundly perceived to build sensible soil profiles. Likewise, the heterogeneity of soil properties can be utilized to make a more adjusted and powerful supplement supply. Likewise, the accuracy preparation plan can stay away from extreme treatment and pointless supplement squander, save costs, yet additionally really safeguard the biological climate, and add to the resulting scientific the board of farmland soil [57]. A few investigations have shown that vegetation restoration is helpful to soil recreation. Vegetation reclamation in upset regions can be accomplished by aloof (unconstrained progression, PR) and dynamic (human-actuated, AR) approaches Contrasted and latent rebuilding, AR speeds up the rebuilding of soil properties. Following 15 years of vegetation reclamation, the variety of bacterial local area re-went to the level before land subsidence [47]. In this way, based on geomorphology renovating and soil redesigning, considering the different level of land corruption in mining regions, the supplement prerequisites of various kinds of harvests and ecological circumstances looked at, to keep the high return of yields, local vegetation screening with various debasement degree was performed. Simultaneously, the restoration of over-the-ground vegetation can advance the reclamation of soil supplement and microbial design security. To summarize, understanding the spatial heterogeneity of soil supplements and microbial local area construction can add to the exact recovery of land, which is helpful to the greatest usage of land assets in the mineral-grain complex region, it is likewise useful to work on the natural climate of coal mining region and advance the reasonable improvement of coal mining region.

Overall, from an ecological perspective, the best arrangement is to gradually reduce coal mining until it is dispensed with. Truth be told, the outcomes propose that coal mining is destructive in terms of dirt created as well as environmental change. In this way, we should not just take advantage of the land assets of the mining region, but aim for complete natural restoration and work on the biological climate of the mining region, in addition to reducing coal mining until the impact brought about by the coal mining is dispensed with.

5. Conclusions

This study examines the disturbance patterns of soil factors and microbial communities in coal and grain production areas, and attempts to understand the impact of subsidence and water accumulation stress on soil characteristics and microbial communities in coal mining subsidence areas with high subsidence levels. The findings unveiled that:

(1) Soil properties and microbial community structure exhibit significant variations across different regions in coal mining subsidence areas with high levels of disturbance. The levels of TN, TP, SOM, and bacterial and fungal diversity in the soil all decline to different extents in areas affected by coal mining subsidence. The distance from the water logging area follows a pattern of initially decreasing and then increasing, with the lowest levels seen in SSP. The soil in the coal mining subsidence area with a high water table is degraded by groundwater and lateral seepage, whereas the soil surrounding the subsidence water area is located at the base of the subsidence slope and is influenced by sedimentation. The presence of coal mining subsidence and water accumulation greatly affects the properties of the surrounding soil.

(2) There are significant disparities in soil properties and microbial community composition between non-waterlogged and waterlogged areas in coal mining subsidence zones with a high water table. The nutrient level and stability of the microbial community structure in coal mining subsidence areas are generally low. However, the SSP shows the lowest level, while the PSP exhibits some improvement. This improvement helps to maintain a high level of soil nutrients and microorganisms, enabling the establishment of a stable wetland habitat. Developing biodiversity and enhancing soil ecosystem stability in areas affected by high-intensity coal mining subsidence is an effective land use strategy.

(3) The presence of Proteobacteria and Firmicutes in bacteria, and Mortierellomycota, Rozellomycota, and Basidiomycota in fungi, differed significantly from the control group (CK) in the coal mining subsidence area. This suggests that the soil microbial communities in the subsidence area were significantly disrupted. It is important to take into account the introduction of these microorganisms during the later reclamation.

(4) The correlation between soil bacterial and fungal diversity, as well as their important populations, and soil properties varied to different extents. The alteration of the microbial community was mostly influenced by soil nutrients and soil water content (SWC).

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