Article

How Does Land Consolidation Affect Soil Fungal Community Structure? Take Heavy Metal Contaminated Areas in Eastern China for Example

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Abstract: Farmland land consolidation can effectively improve the quality of farmland soil and the agricultural production level, and can effectively guarantee farmland ecology and food security, which has been widely used in the world. A large number of studies have shown that farmland consolidation has certain adjustments to the basic physical and chemical properties of soil and the content of heavy metals. As a key indicator of soil quality and ecological conditions, soil microorganisms play an important role in soil pollution restoration and the promotion of crop growth. However, there are few domestic and foreign studies on how farmland consolidation affects soil microbial properties, and there are no related reports on the mechanism of action between them, which is a blank in the field of agricultural land consolidation and soil microecology, especially in heavy metal contaminated areas. Therefore, we used the DNA sequence technology to compare fungal community structure in farmlands with and without consolidation in heavy metal contaminated areas. Our results showed that (1) farmland consolidation had a significant impact on soil microbial characteristics, which were mainly manifested as changes in microbial biomass, microbial diversity and community structure. (2) Farmland consolidation had an indirect impact on soil fungal community structure by adjusting the soil physical and chemical properties. (3) The impact of heavy metals on the fungal community structure varied significantly under different levels of heavy metal pollution in farmland consolidation areas. When the pollution was at the highest level, there existed 7 fungus genera showing a strong tolerance to heavy metals and consuming a lot of soil nutrients, of which were *Melanospora*, *Pseudeurotium*, *Guehomyces*, *Schizothecium*, *Gibberella*, *Myrothecium*, and *Neurospora*. In this study, an analytical method was proposed to analyze the effects of farmland consolidation on soil fungi, and the mechanism was discussed from two aspects—soil physical and chemical properties, and heavy metal content. The results shed some light on farmland consolidation, cultivated land quality evaluation and territorial space ecological restoration.

Keywords: farmland consolidation; cultivated land quality; soil basic physical and chemical properties; heavy metals; fungi

1. Introduction

As one of the most effective land management measures to improve agricultural production and the ecological environment, farmland consolidation is widely used in most countries in the world to consolidate scattered land and improve agricultural facilities and soil quality [1–3]. Nowadays, in order to cope with the increasing risks of farmland, researchers are exploring the multifunctional potential of farmland consolidation

to solve many development problems, such as agriculture, nature, landscape, economy and tourism [4,5]. However, farmland consolidation also affects the physical and chemical properties of farmland soil and microbial communities, especially in terms of soil fungal diversity [5,6]. In order to promote the strategic deployment of China's ecological civilization construction, the development direction of farmland consolidation will become more ecological. However, the soil fungus community, which is profoundly affected by farmland consolidation, has not received sufficient attention and needs to be paid attention to in future research and practice. Under the current situation, researchers need to explore how to improve soil quality and increase farmland productivity by adopting appropriate farmland consolidation methods, scientifically evaluate the impact of farmland consolidation on soil fungal community structures, and extract microbial indicators that have the significance of indicating the farmland quality indicators. This will be an important issue in the field of global farmland improvement and farmland ecological environmental protection.

Soil fungi are efficient drivers of material and energy circulation in the soil environment. They have made a huge contribution to creating a healthy and stable soil ecological environment, and at the same time, they have also improved the diversity of regional ecosystem service values and ecological functions [7]. As an important member of soil microorganisms, soil fungi are also an important part of the earth's ecosystem. They mainly affect global climate change through the regulation of CO_2, CH_4, N_2O and other greenhouse gases [8]. There have been many international studies on the association of soil fungal communities with climate change, and it has also been confirmed that the regulation of soil microbes on carbon, nitrogen, phosphorus and other element cycles plays a vital role in the feedback of climate change [9,10]. In addition, soil fungi can promote or inhibit the growth and development of plants through their own life activities, thereby affecting the number and diversity of plant community structures and regulating the performance of the regional ecological environment [11,12]. Soil fungi, especially mycorrhizal fungi, can form symbiosis with plant roots to improve the ability of plants to resist drought and diseases, and provide plants with essential nutrients such as N, P, and K [13]. It can be seen that the types and functions of soil fungi have a strong regulatory effect on the diversity of plant communities, and will have a greater impact on the evolution of regional ecosystems. However, the soil fungal community structure is extremely susceptible to the influence of the soil environment. As a human activity, farmland consolidation has a strong disturbance to the physical and chemical properties of the soil, which will directly or indirectly affect the soil fungal community and change its ecological function. Therefore, the impact of farmland consolidation on soil fungal communities will be important content in future research fields, such as land use, environmental management, and microbial diversity.

The main purpose of this study is to clarify the influence mechanism of farmland consolidation on the structure of the soil fungus community, to explore and analyze the effect of different farmland consolidation measures on the structure of the soil fungus community, and to reveal the main environmental factors that affect the structure of the soil fungus community in heavy metal contaminated areas. By exploring the influence mechanism of different farmland consolidation measures on the physical and chemical properties of soil, the pollution levels of the heavy metals soil, soil fungal community structure and functional diversity, and the corresponding feedback mechanism, it can provide certain guidance for improving the efficiency of farmland consolidation. Through the analysis of soil fungal communities under different heavy metal pollution levels in the farmland consolidation area, to identify specific strains with heavy metal tolerance, it can provide a scientific basis for the bioremediation of contaminated soil in the remediation area, and the selection of farmland quality evaluation indicators.

2. Framework and Data Collection

2.1. Research Framework

This research was based on three major theories: sustainable development theory, restoration ecology theory, and microbial ecology theory. This study fully explains the

impact mechanism of agricultural land consolidation on soil fungi from three aspects: the impact of agricultural land consolidation on soil's physical and chemical properties, the interaction mechanism between soil's basic physical and chemical properties and fungal communities, and the interaction mechanism between heavy metals soil and soil with fungal communities (Figure 1). There was a close relationship between farmland consolidation and soil fungal communities, and it could be effectively reflected from the analysis of the basic physical and chemical properties of soil, heavy metal soil pollution and fungal community structure diversity. This research served as a useful exploration of farmland consolidation and soil micro-ecology, which could provide a certain reference for related research in the future.
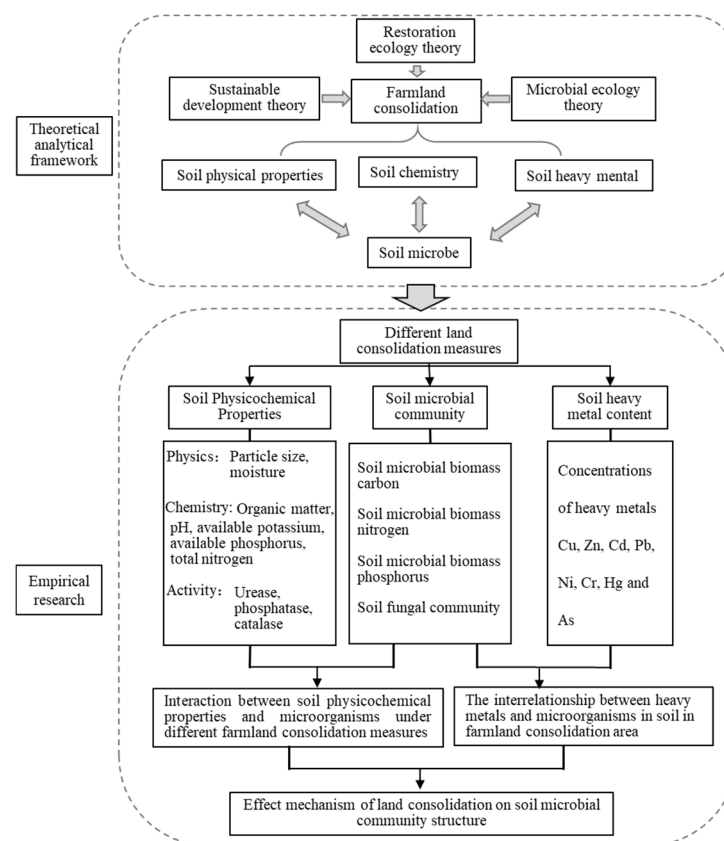
Figure 1. Research framework.

2.2. Study Area

The study area of this article is a county in the east coast of China. The total area of the county is about 500 square kilometers, of which the water area accounts for about 15% and the land area accounts for about 85%. The region belongs to the southeast Asian monsoon region, with four distinct seasons and a mild climate. The annual average temperature is 15.6 °C, the annual average rainfall is 1155.7 mm, the annual average relative humidity is 65%, the annual average sunshine is about 2000 h, and the frost-free period is 236 days. The county has an advantageous geographical location and a pleasant climate, making it an important grain producing area. However, fragmentation of arable land, incomplete agricultural facilities, degradation of soil fertility, and soil pollution are still serious problems in the process of sustainable agricultural development in this region. Therefore, farmland consolidation is an important task in recent years to improve the agricultural production and ecological soil environment in this area.

2.3. Soil Collection and Analysis

2.3.1. Soil Collection

In September 2018, 40 farmland consolidation areas were randomly selected in the study area. All samples have 4 to 8 years of rice planting history after farmland consolidation, numbered A01-A40. At the same time, 10 plots of farmland without farmland consolidation were randomly selected around the farmland consolidation area. All samples have decades of rice planting history, numbered B01-B10. The sample points in the non-agricultural land consolidation area were next to the farmland consolidation area, and had similar soil properties to the farmland consolidation area. These non-agricultural land consolidation areas could be used as effective controls for soil samples in agricultural land consolidation areas. The soil samples in the farmland consolidation area were grouped according to the adopted farmland consolidation measures. The specific grouping information was shown in Table 1.

Table 1. Layout of sample points.

Land Consolidation Measures	Sample	Main Methods	Sum
Building ditches	A04 A05 A06 A07 A08 A10 A11 A14 A15 A19 A21 A23 A24 A25 A27 A28 A30 A33 A35 A36 A37 A38 A39 A40	Digging and constructing waterways in the field to facilitate irrigation or drainage.	24
Merging plots	A01 A02 A04 A05 A06 A08 A13 A14 A18 A20 A24 A25 A26 A28 A29 A30 A31 A32 A34 A37 A39	Including plowing, deep loosening, harrowing, drowning, suppression, land levelling, ridging, and upsetting.	21
Land levelling	A02 A03 A04 A05 A06 A07 A08 A09 A12 A13 A14 A15 A16 A17 A20 A22 A23 A24 A25 A27 A28 A30 A33 A35 A37 A38 A39	Through ownership adjustment and mechanical means, the scattered plots will be merged into large plots of cultivated land for unified farming management.	27
Applying organic fertilizers	A02 A03 A05 A06 A08 A10 A11 A14 A15 A16 A17 A18 A19 A21 A22 A23 A24 A25 A28 A30 A32 A36 A37 A38 A39 A40 B01	In the study area, organic fertilizers were mainly applied in addition to chemical fertilizers.	27
Comprehensive improvement	A05 A06 A08 A14 A24 A25 A28 A30 A37 A39	Simultaneously implement the four measures of building ditches, merging land, levelling land and applying organic fertilizer.	10
Non-agricultural land consolidation	B01 B02 B03 B04 B05 B06 B07 B08 B09 B10	It is a traditional farming method and mainly uses chemical fertilizers.	10

In each sample area, a 10*10 m plot was divided from the area away from the road, and the surface soil (0–15 cm) was taken, according to the five-point method, and mixed into a soil sample to represent the sample area [6,14]. Soil samples from all 50 sampling locations were removed from roots, rocks, plant residues, animal remains and other debris with tweezers in sterilized stainless steel vessels. Twenty grams of soil for each sample were transported back to the laboratory in dry ice and stored at −80 °C for DNA extraction [15]. At the same time, 500 g of soil were stored for each sample in a sealed bag and transported back to the laboratory for basic physical and chemical properties and heavy metal tests. After the soil was homogenized, each sample was subjected to 3 times of DNA extraction and soil environmental variable detection.

2.3.2. Soil Basic Physical and Chemical Properties Test

The pH value of the soil solution was measured at 1:2.5 (soil: water) with a pH meter. The soil moisture content (SW) was measured by drying in an oven at 105 °C for 12 h [16]. Soil organic matter (OM) was calculated by measuring the total organic carbon content with a total organic carbon analyzer (BOCS301, Shimadzu, Japan). Soil total nitrogen (TN), soil available phosphorus (AP), soil total phosphorus (TP), and soil available potassium (AK) were respectively measured by automatic Kjeldahl nitrogen analyzer (K9860, Haineng,

Qingdao, China), flame photometer and spectrophotometer photometric measurement [17]. Soil enzyme activities such as catalase, urease, and phosphatase activities were determined by sodium phenate, sodium phenol-sodium hypochlorite colorimetry and phenyl disodium phosphate colorimetry [18]. These tests were repeated three times for each sample.

2.3.3. Heavy Metal Soil Content Test

After the soil sample was extracted by HCl-HNO_3-HF-$HClO_4$, the concentration of Cu, Zn, Cr, Cd, Pb and Ni in the soil was measured by an inductively coupled plasma source mass spectrometer (Agilent 7800, California, CA, USA) [19]. The specific method was to add 3 mL of 37% HCl, 1 mL of 65% HNO_3, 6 mL of 65% HF and 0.5 mL of 65% $HClO_4$ to a 0.1 g soil sample for digestion. The digestion solution was evaporated to near dryness and dissolved in 1.0 mL of 65% HNO_3, and then 20 mL of deionized water was added. In addition, the concentration of Hg and As in the soil was pretreated with aqua regia according to the national standard (GB 22105-2008), and then measured by an atomic fluorescence spectrophotometer (AF-630, BFRL, Beijing, China) [19]. This experiment was repeated 3 times and a blank control was used to ensure the quality of the test.

2.3.4. Soil Microbial Properties Determination

(1) Soil microbial biomass determination

Soil microbial biomass usually included soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass phosphorus (MBP), etc., and it was mainly determined by chloroform fumigation extraction method [20]. We fumigated 10 g of sieved soil and the blank control group with de-ethanol chloroform, added K_2SO_4 for shaking extraction, and filtered after 24 h. For microbial biomass carbon, after adding $K_2Cr_2O_7$ and H_2SO_4 solution to the extract to boil, adding $FeSO_4 \cdot 7H_2O$ after titration, the coefficient conversion was obtained. Microbial biomass nitrogen was processed by adding $CuSO_4$ and concentrated H_2SO_4 to the extract, then adding NaOH and connecting a distillation nitrogen analyzer to absorb the released NH_3, and the result was calculated by coefficient conversion. The microbial biomass phosphorus was measured in a spectrophotometer by using $NaHCO_3$ and KH_2PO_4.

(2) DNA extraction and sequencing analysis

According to the instructions of the FastDNA SPIN kit (MP Biomedicals, Santa Ana, CA, USA), the microbial DNA for PCR amplification was extracted from 0.5 g of soil sample. In addition, a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used to measure the concentration and quality of the extracted DNA, and the extracted DNA was stored in a refrigerator at $-20\ ^\circ C$ for later use. The ITS1 region of fungi was amplified by primers ITS1F and ITS2R [21].

PCR used a 20 μL reaction system, including MdNTPs, FastPfu Buffer, FastPfu Polymerase, primers, BSA, DNA template, and finally added pure water to 20 μL. We performed the following amplification procedures with ABI GeneAmp$^\circledR$ 9700 PCR machine: pre-denaturation at $95\ ^\circ C$ for 3 min; at $95\ ^\circ C$ for 30 s, $60\ ^\circ C$ for 30 s, $72\ ^\circ C$ for 45 s, and repeat 10 cycles; extend at $72\ ^\circ C$ for 10 min; ran at $10\ ^\circ C$ for 30 min. After the amplified products were electrophoresed on a 2% agarose gel, the PCR mixed products were recovered with a gel extraction kit (Omega, Norcross, GA, USA).

We used the Illumina HiSeq4000 platform for sequencing and microbial community analysis [22]. The sequencing data were analyzed on the Illumina HiSeq4000, and the original DNA sequencing data were processed by the Quantitative Insights Into Microbial Ecology 2 platform (QIIME 2) [23]. High-quality sequences with 97% similarity were classified into operational taxonomic units (OTUs) by Usearch 7.1, and sequences that occurred only once were removed before clustering the sequences in OTUs to improve the accuracy of diversity assessment.

2.4. Statistic Analysis

The microbial alpha diversity index could reflect the richness and diversity of microbial communities, including Shannon, Simpsoneven, Simpson and Chao 1, etc., which were all evaluated by the mothur software package (version v.1.30.1) [24]. The composition of the microbial community was mainly displayed in the form of a column chart and a Heatmap chart through the vegan package of the R language. The difference of microbial communities among different samples was mainly analyzed by QIIME calculation of β diversity index. The influencing variables of the differences in the composition of soil microbial communities were processed and discriminated by PLS-DA (Partial Least Squares Discriminant Analysis). Mantel test was used to test the correlation between a single or a group of environmental factors and OTUs, to characterize the correlation between environmental factors and the entire microbial community. The correlation between environmental factors and soil microbial species was mainly analyzed by Spearman using R language and displayed in the form of Heatmap. The functional composition of soil microorganisms was mainly used to predict the function of amplicon sequencing data of fungi through Funguild software. These were commonly used and scientifically effective analysis methods in the field of bioinformatics [22].

For the assessment of heavy metal pollution levels, the geological accumulation index (GI) proposed by Muller was used, and this method had been widely used in paddy soil [25]. In addition, the Nerome Comprehensive Index (NI) was a common method based on GI to reflect the level of heavy metal pollution [26]. Therefore, the level of heavy metal pollution in the soil could be effectively evaluated by combining the two methods of GI and NI. GI was a geochemical standard for determining the pollution level of a single heavy metal in the soil by comparing it with the pre-industrial level. The formula was as follows:

$$\text{GI}_i = log_2\left(\frac{C_i}{1.5B_i}\right) \tag{1}$$

C_i represented the concentration of a single heavy metal in the soil collected from sample I, and B_i represented the geochemical background value of a single heavy metal in the sample in this area. In addition, existing studies had proved that the influence of the external environment and human activities on the fluctuation of metal content could be expressed by a coefficient of 1.5 [27]. Next, NI could be determined based on the results of GI, and a reasonable and comprehensive assessment of pollution could be obtained. The formula was as follows:

$$\text{NI}_i = \sqrt{\frac{GI_{iave}^2 + GI_{imax}^2}{2}} \tag{2}$$

GI_{iave} and GI_{imax} showed the average and maximum GI_i values of eight heavy metals, respectively. According to the NI value, the level of heavy metal pollution could be divided into severe level (SL, NI > 3), moderate level (ML, 2 < NI ≤ 3), light level (LL, 1 < NI ≤ 2) and clean level (CL, NI ≤ 1) [28].

3. Results and Discussion

3.1. Effect of Land Consolidation on Soil Fungal Community

3.1.1. Changes in Soil Fungal Diversity

Analysis of α Diversity of Fungal Community

The soil samples in the study area obtained 2,794,117 effective sequences after the amplification of the ITS1F_ITS2R sequence region and optimization of quality control, with an average length of about 258 bp, aggregated into 5617 OTUs. By making the Shannon dilution curve (Figure 2), a curve that tends to be flat was obtained. This showed that the amount of sequencing data in this experiment was appropriate, which was sufficient to reflect the vast majority of fungal diversity information in the soil sample. If the amount of sequencing data continued to increase, only a very small number of new fungal

strains could be generated. This showed that the sequencing data in this experiment were reasonable and could meet the needs of accurately reflecting the diversity of fungi.
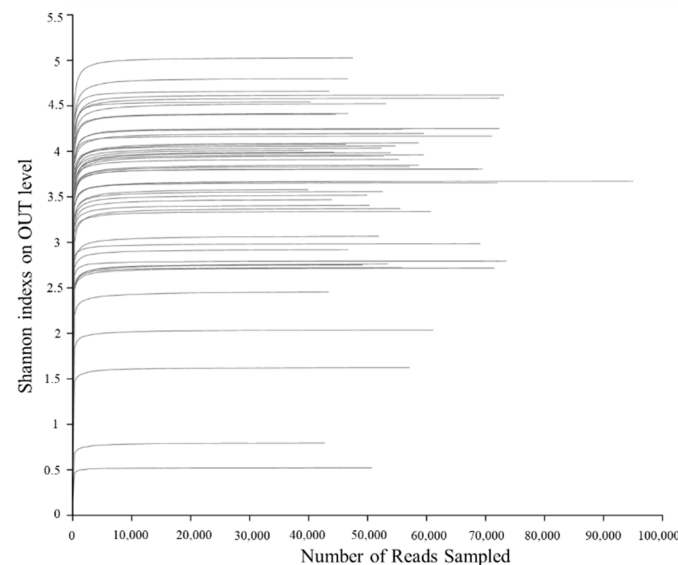
Figure 2. Shannon dilution curves of different samples.

After measuring the fungal community alpha diversity index on soil samples, Spearman correlation analysis was used to calculate the correlation and significant relationship between the alpha diversity index of different grouping soil samples (Table 2). The fungal alpha diversity index mainly included Sobs and Chao, reflecting community richness indexes, Simpsoneven and Shannoneven, reflecting community evenness indexes, Shannon and Invsimpson, reflecting community diversity indexes, and Coverage, reflecting community coverage. In the study area, the soil fungal community richness indexes, Sobs and Chao, of cultivated land that had been renovated on farmland were significantly higher than those in the non-agricultural land consolidation area at the $p < 0.05$ level. At the same time, the Chao index of the soil fungal community in the land consolidation area of the combined land was significantly lower than that of other agricultural land consolidation areas. This showed that the effect of combined plots on the improvement of fungal community diversity was relatively inefficient compared with other farmland improvement measures. For Simpsoneven and Shannoneven, which reflected the community evenness index, the soil fungal community evenness index on the plots under comprehensive improvement was significantly lower than that in other areas. It was inferred from this that comprehensive improvement measures had a greater impact on the relative species richness of the fungal community, resulting in greater differences in the evenness of the fungal community in the habitat. In the study area, the Shannon diversity index of the soil fungus community in the comprehensive improvement area was significantly lower than that in other areas, while the soil fungal community diversity index, Invsimpson, in the non-agricultural land consolidation area, was significantly higher than that in the farmland consolidation area. This showed that the fungal community was more susceptible to the disturbance of farmland consolidation activities, thereby reducing its community diversity. In addition, the Coverage Index of the soil fungus community in the study area was close to 0.99, indicating that the experimental data were highly reliable and provide a guarantee for the scientificity of the research results.

Table 2. Table of soil fungal diversity indicators under different farmland consolidation.

| Land Consolidation Measures	The Community Richness Index		The Community Evenness Index		The Community Diversity Index		The Community Coverage Coverage
	Sobs	Chao	Shannoneven	Simpsoneven	Shannon	Invsimpson	
Comprehensive improvement	4312.00 ± 452.33 a	5866.69 ± 523.61 a	0.89 ± 0.01 a	0.17 ± 0.02 a	7.44 ± 0.12 a	730.73 ± 118.61 a	0.95 ± 0.01 a
Applying organic fertilizers	4318.92 ± 447.21 a	5768.07 ± 530.29 a	0.88 ± 0.01 a	0.15 ± 0.04 a	7.36 ± 0.16 a	658.19 ± 189.31 a	0.96 ± 0.01 a
Building ditches	4370.70 ± 443.19 a	5808.02 ± 493.61 a	0.88 ± 0.01 a	0.16 ± 0.03 a	7.39 ± 0.14 a	693.77 ± 161.98 a	0.95 ± 0.01 a
Merging plots	4349.68 ± 440.11 a	5823.19 ± 528.11 a	0.88 ± 0.01 a	0.15 ± 0.04 a	7.38 ± 0.15 a	675.83 ± 176.20 a	0.96 ± 0.01 a
Land levelling	4405.47 ± 396.52 a	5963.16 ± 483.81 a	0.88 ± 0.01 a	0.16 ± 0.04 a	7.39 ± 0.17 a	696.92 ± 202.67 a	0.96 ± 0.01 a
Non-agricultural land consolidation	3338.20 ± 675.30 b	4348.85 ± 922.49 b	0.87 ± 0.01 a	0.13 ± 0.04 a	7.01 ± 0.25 b	439.17 ± 166.72 b	0.96 ± 0.01 a

Note: The average value in the table is presented in the form of mean ± standard deviation. Different lowercase letters in the same column represent significant differences at the $p < 0.05$ level.

From the above analysis, it could be seen that the analysis of the Shannon dilution curve and Coverage index proved that the data of this experiment were of high quality and strong operability. The measurement of the fungal community alpha diversity index confirmed that farmland consolidation had a higher effect on improving the richness of soil fungal communities, but it significantly reduced the evenness and diversity of fungal communities.

Analysis of β Diversity of Fungal Community

β diversity analysis was usually used to compare and analyze the diversity of microbial communities between different sample groups, that was, to analyze the differences between samples. This was mainly to measure the sample distance through the Bray–Curtis distance algorithm to evaluate the abundance of information of community species and the evolutionary relationship between samples, so as to effectively reflect the significant differences in microbial communities between different sample groups. PCoA (Principal Co-ordinates analysis) was the principal coordinate analysis. It was a commonly used method of β diversity analysis. The R language was used to sort the eigenvectors and eigenvalues, and the most important eigenvalues were selected for representation in the coordinate system. The principal components that had an important effect on the composition of the sample microbial community by way of dimensionality reduction were identified.

In this study, all samples were divided into two groups: farmland consolidation areas and non-agricultural land consolidation areas for PCoA of fungal communities (Figure 3). It could be seen from the figure that the fungal communities in the two soil samples showed different clusters, which indicated that there were obvious differences in the soil fungal community structure between the groups. PC1 and PC2 had an explanation degree of about 45% for the differences in the composition of the sample fungal communities. This could be understood, as farmland consolidation affected the changes in the fungal community structures by changing the physical and chemical properties of the soil, so farmland consolidation played the most important role.
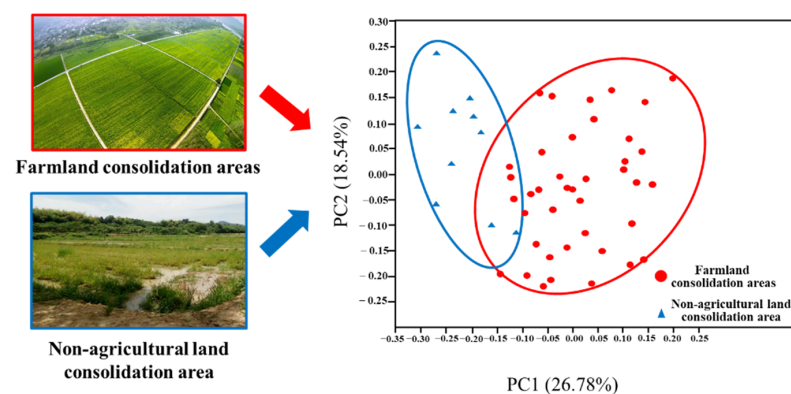

Figure 3. The PCoA map of fungal communities with OTU levels of samples between different groups.

3.1.2. Changes in Soil Fungal Community Structure

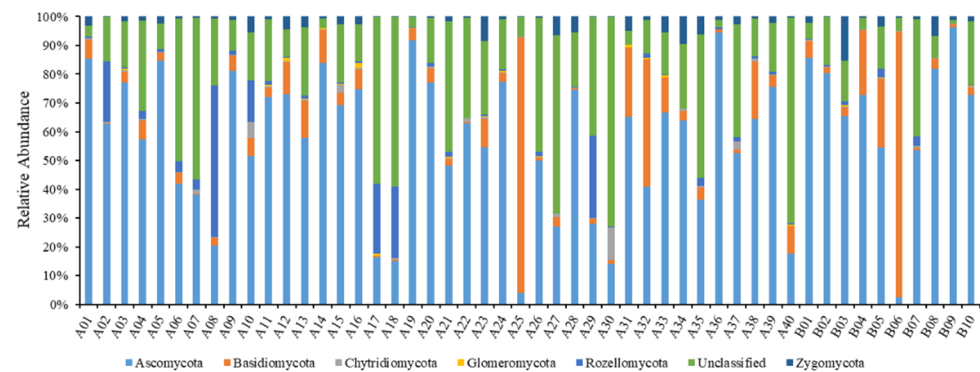Analysis on the Changes of Fungal Community at the Phylum Level

There were a total of 50 samples in the study area. The 2,794,117 high-quality soil fungal sequences obtained after the tests were aggregated into 5617 fungal OTUs. These high-quality sequences could be divided into 6 known fungal phyla, namely Ascomycota, Basidiomycota, Rozellomycota, Zygomycota, Chytridiomycota, and Glomeromycota. The number of effective sequences in these phyla was 2,120,951, accounting for about 76% of the total; the number of OTUs was 1805, accounting for about 32% of the total. Among them, there were 4 phyla with a relative abundance greater than 1%. The sequence number and OTU number of Ascomycota were 1,663,154 and 1268, accounting for 59.52% and 22.57% respectively; the sequence number and OTU number of Basidiomycota were 254,664 and 272, accounting for approximately 9.11% and 4.84%, respectively; Rozellomycota's sequence number and OTU number were 107,670 and 13, accounting for approximately 3.85% and 0.23%, respectively; Zygomycota sequence number and OTU number were 67,111 and 82, respectively. The proportions were about 2.41% and 1.46% respectively. In addition, the sequence number and OTU number of Chytridiomycota were 20,555 and 114, respectively, accounting for approximately 0.74% and 2.03%, respectively. The sequence number and OTU number of Glomeromycota were 7797 and 56, respectively, accounting for about 0.28% and 1.00% respectively (Figure 4).
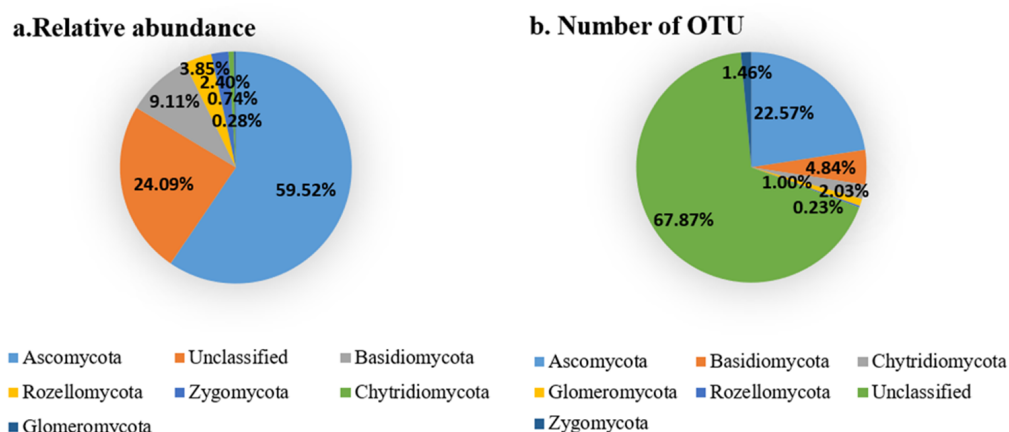
Figure 4. The relative abundance and OTU composition of soil fungi in the study area.

The relative abundance of soil fungi in the extracted samples was compared (Figure 5). The results showed that the average relative abundance of Rozellomycota, Chytridiomycota, and Glomeromycota in all grouped soils in the farmland consolidation area (samples A01–A40) was higher than that of the non-agricultural land consolidation area (samples B01–B10), but the average relative abundance of Ascomycota, Basidiomycota and Zygomycota was lower than that of the non-agricultural land consolidation area. It could be seen that the farmland consolidation in the study area could increase the relative abundance of the three types of mycota, but also reduced the relative abundance of the remaining three types of mycota.

All soil samples were divided into six groups: building ditches, land levelling, merging plots, the applying of organic fertilizer, comprehensive improvement, and non-agricultural land consolidation areas. The top 20 dominant fungal genera with relative abundance in each group of soil samples were extracted for comparative analysis (Figure 6). A total of 2,794,117 high-quality soil fungal sequences from 50 samples in the study area could be divided into 463 fungal genera. Among them, the dominant genera, with a relative abundance greater than 1%, include *Gibberella*, *Zopfiella*, *Schizothecium*, *Pseudeurotium*, *Fusarium*, *Guehomyces*, *Bolbitius*, *Acremonium*, *Mortierella*, *Curvularia*, *Talaromyces*, *Myrothecium*, *Pyrenochaetopsis*, *Melanospora*, etc.

Figure 5. The relative abundance of soil fungi in all samples in the study area.

Analysis on the Changes of Fungal Community at the Genus Level
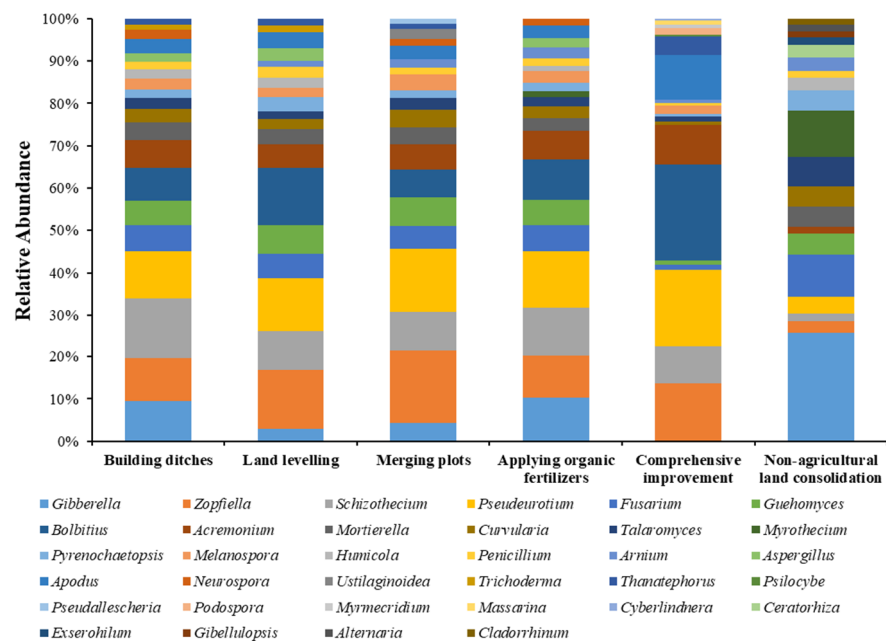
Figure 6. Relative abundance of dominant fungal genera in all soil sample groups in the study area.

The number of effective sequences of these dominant fungi was 1,006,999, accounting for about 36% of the total. The average relative abundance of *Gibberella* was the highest in the collected soil samples, about 5.25%, and it accounted for the largest proportion, of 12.17%, in farmland soil in non-agricultural land consolidation areas, and the lowest proportion in farmland soil under comprehensive improvement was 0.21%. As a harmful fungus that causes the rice bakanae disease, *Gibberella* could cause rice to grow excessively and reduce grain yield and quality. In addition, harmful fungi such as *Fusarium* and *Curvularia*, which were likely to cause rice head blight and fusarium wilt, had the highest relative abundance (4.69%, 2.20%) in non-agricultural land consolidation areas, but they had the lowest relative abundance (0.83%, 0.41%) in comprehensive improvement areas. It could be seen that the relative abundance of *Gibberella*, *Fusarium*, *Curvularia* and other harmful fungi in farmland consolidation areas was significantly lower than that in non-agricultural land consolidation areas. It could be seen that farmland consolidation could effectively control the relative abundance of such fungi to improve soil quality.

Analysis of Changes in Fungal Community Function

In order to analyze the differences in soil fungal functions between different groups, the fungus community function was predicted, classified and analyzed by relative abundance

through Funguild software (Figure 7). The soil fungi in the study area could be divided into a pathotroph, that obtains nutrients by destroying host cells, symbiotroph, that cooperates with the host and exchanges nutrients, saprotroph, that degrades dead cells in the host to obtain nutrients, and various transitional types formed by these three types [29]. The study area was dominated by saprophytic trophic types, with a relative abundance of 28.30%~57.72%. The area where comprehensive improvement was implemented had the highest value. The relative abundance of the remaining four consolidation types was relatively close, while the non-agricultural land consolidation area had the lowest. Since saprophytic trophic fungi could produce a large amount of hydrolysis and oxidase to promote the decomposition of organic matter and provide nutrients for plant growth, farmland consolidation could significantly increase the relative abundance of saprotrophic fungi in soil to improve soil fertility [30]. In addition, the relative abundance of pathotrophic fungi in the non-agricultural land consolidation area was significantly higher than that in the farmland consolidation area, which was 19.68%, while the farmland consolidation area was only 3.97% to 7.61%. Pathotrophic fungi were dominated by Fusarium, which could secrete various toxins to cause plant wilt or tissue necrosis, and might pose a serious threat of organ failure to the eater [31]. Farmland consolidation effectively reduced the relative abundance of pathological trophic fungi by affecting the soil environment, which was of great significance to soil health and food security.
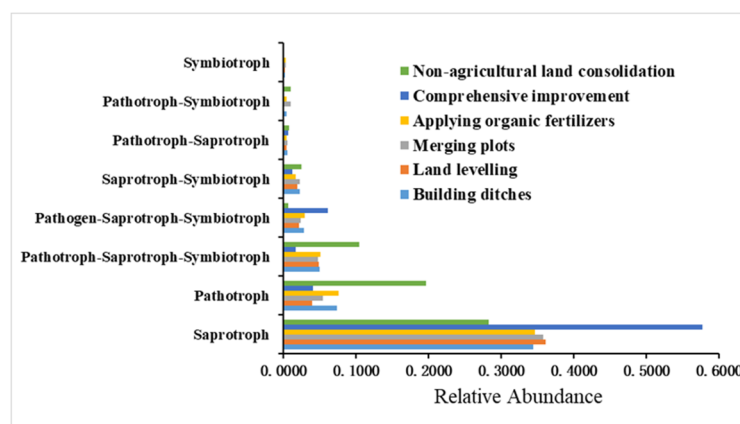
Figure 7. Functional types and relative abundance of fungal communities in soil samples between different groups.

3.2. Farmland Consolidation Regulates the Basic Physical and Chemical Properties of Soil and Its Mechanism of Action on Fungi

3.2.1. Farmland Consolidation Promotes Changes in Basic Physical and Chemical Properties of Soil

Soil Physical Properties

The soil physical properties in the study area mainly included soil particle size and soil moisture content, which were the core content of current soil physics research. Soil particle size would directly affect the distribution of soil pores, and had a significant impact on soil aeration and water holding capacity. A reasonable particle size distribution was conducive to the healthy growth of crop roots and effectively increased food production [32]. Soil moisture was an important driving force necessary for the soil's physical processes, and was affected by natural factors such as vegetation, topography, climate, and human activities at different scales [33]. At the same time, however, it also affected the nutrient cycle and energy conversion in the soil environment and directly affected the growth and development of crops.

The soil particle size in the study area ranged from 10.16 to 30.42 μm, and the average soil particle size in the non-agricultural land consolidation area was the smallest, which was significantly lower than that in the farmland consolidation area (Figure 8). Studies had shown that when the soil particle size in paddy soil was less than 20μm, the content of the

soil's organic matter would be significantly reduced, and when the soil particle size was in the range of 20–250 μm, it would play a good carbon sink function [34]. This was mainly due to the fact that soil particles with a larger particle size had better permeability, and the positive charge carried by themselves was easily combined with the humus carrying negative charges. It was also easier to be decomposed by soil microorganisms to increase the organic matter content in the soil [35]. In this study area, the average soil particle size in each farmland consolidation group was greater than 20 μm and the distribution was relatively uniform, while the average soil particle size in non-agricultural land consolidation was only 17.48 μm. It could be seen that farmland consolidation could change the distribution of soil particle size, through measures such as project implementation and soil fertilization, which had a certain driving effect on the change of soil properties. In addition, the soil moisture content in the study area ranged from 20.70% to 54.18%. The comprehensive improvement area had the highest average soil moisture content (47.00%), followed by the average soil moisture content of the plots where construction ditches were implemented (43.87%). The non-agricultural land consolidation area had the lowest average soil moisture content (30.39%). It could be seen that the improvement of farmland irrigation facilities and the optimization of the soil mechanical structure could effectively increase soil moisture content and provide a sufficient water supply for crop growth [36].
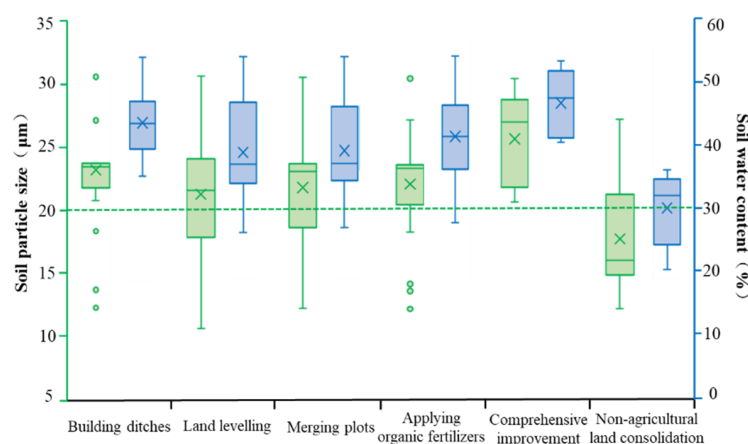
Figure 8. Distribution of soil particle size (green) and soil moisture (blue) in the study area.

Soil Chemistry

Soil chemistry is an important branch of soil science, including soil pH, organic matter, available potassium, available phosphorus and other indicators. It plays a key role in the process of soil productivity, self-purification capacity, carbon emissions and nutrient balance [37]. The current frontier research field of soil chemistry is the cross-discipline of soil chemistry and microbiology, and soil microbes have played an important role in the development of soil chemistry research.

The pH value of the soil in the study area ranged from 6.15 to 8.30. The average soil pH value in the non-agricultural land consolidation area was the highest and the value fluctuated the most, which was significantly higher than that in the areas where construction ditches, combined plots, organic fertilizers and comprehensive improvement were implemented, in which the soil pH was close to neutral (Table 3). This was because measures such as building ditches and merging plots could effectively promote the flow of water in the farmland. At the same time, the application of organic fertilizers could reduce the input of inorganic fertilizers, which could effectively adjust the pH of the soil [38]. For soil nutrients, the content ranges of organic matter, available phosphorus, available potassium, and total nitrogen were 16.00~70.40 g/kg, 8.56~330.79 mg/kg, 14.45~80.25 μg/mL and 0.93~3.75 g/kg, respectively. The average soil nutrients in the non-agricultural land consolidation area was the lowest, and it was significantly lower than that in the farmland consolidation area. The application of organic fertilizer and comprehensive improvement

areas had an especially higher soil nutrient content. It could be seen that the application of organic fertilizers in farmland consolidation areas effectively improved soil nutrients, and the construction of ditches accelerated the flow of water to promote nutrient cycling, thereby creating a healthy soil environment to accelerate the promotion of microorganisms in soil nutrient cycling to achieve a virtuous circle.

Table 3. The chemical properties of soil (0–15 cm) between different groups in the study area.

Group	pH	Organic Matter(g/kg)	Available Phosphorus (mg/kg)	Available Potassium (µg/mL)	Total Nitrogen (g/kg)
Building ditches (*n* = 24)	7.09 ± 0.63 b	46.63 ± 14.22 b	90.78 ± 83.78 a	29.65 ± 13.31 a	2.41 ± 0.73 b
Land levelling (*n* = 27)	7.23 ± 0.63 a	41.97 ± 15.33 b	72.85 ± 54.01 b	28.99 ± 12.00 a	2.20 ± 0.77 b
Merging plots (*n* = 21)	7.11 ± 0.57 b	41.52 ± 15.30 b	70.24 ± 57.77 b	28.60 ± 15.25 a	2.23 ± 0.77 b
Applying organic fertilizers (*n* = 27)	7.11 ± 0.64 b	52.49 ± 11.59 a	93.67 ± 84.50 a	31.38 ± 14.57 a	2.51 ± 0.64 b
Comprehensive improvement (*n* = 10)	6.93 ± 0.51 b	57.98 ± 11.66 a	79.80 ± 58.37 b	37.78 ± 7.61 a	3.08 ± 0.45 a
Non-agricultural land consolidation (*n* = 10)	7.38 ± 0.71 a	30.21 ± 9.80 c	53.43 ± 37.13 c	24.00 ± 14.93 b	1.69 ± 0.45 c

Note: The average value in the table is presented in the form of mean ± standard deviation. Different lowercase letters in the same column represent significant differences at the $p < 0.05$ level.

Soil Enzyme Activity

Soil enzymes are an important driving force for the promotion of soil biochemical cycles. Not only can they sense changes in soil properties, but their activity represents the capacity of the soil's nutrient supply, which is a key indicator of soil quality [39]. Among them, catalase is mainly involved in the chemical process of soil redox, which can effectively characterize the content of the soil's organic matter and the degree of soil decay [40]. Phosphatase and urease are involved in soil's nitrogen and phosphorus cycles, respectively, and are important indicators to characterize soil's nitrogen and phosphorus conversion capacity [41]. At the same time, soil enzyme activities can also interact with soil microbial communities. For example, soil enzymes can participate in the degradation of microbial residues, and changes in the structure of microbial communities will also affect soil enzyme activities, thereby affecting the decomposition of soil's organic matter and the process of nutrient cycling [42]. In addition, fertilization and engineering measures will also have a certain impact on soil enzyme activity. Studies had shown that applying nitrogen fertilizer and increasing soil moisture could significantly increase soil enzyme activity [43]. Therefore, this study selected catalase, phosphatase and urease for group determination, and tried to analyze the effect of farmland improvement on soil enzymes.

According to the test results (Table 4), the soil catalase activity in the study area ranged from 125.45 to 276.90 mg/g, and there was no significant difference between the groups. However, the soil catalase activity in the non-agricultural land consolidation area was the lowest, with an average of 183.58 mg/g. The soil phosphatase activity ranged from 2.80 to 45.20 mg/g. Among them, the soil phosphatase activity of comprehensive improvement was the highest, and the soil phosphatase activity of farmland consolidation areas was significantly higher than that of non-agricultural land consolidation areas. The soil urease activity ranged from 0.01 to 0.98 mg/g, and there was no significant difference among the groups. However, the soil urease activity in the non-agricultural land consolidation area was the lowest, and the soil urease activity in the comprehensive improvement area was the highest. Farmland consolidation effectively improved the soil environment of farmland by adjusting the soil's pH, improving soil nutrients, and accelerating soil water circulation, which has a greater promotion effect on the increase of soil enzyme activity [44,45].

Table 4. Three soil enzyme activities in the study area.

| Group	Catalase (mg/g)	Phosphatase (mg/g)	Urease (mg/g)
Building ditches	207.66 ± 38.30 a	16.017 ± 8.44 a	0.25 ± 0.16 a
Land levelling	203.62 ± 38.48 a	17.35 ± 10.65 a	0.28 ± 0.22 a
Merging plots	201.61 ± 32.37 a	15.28 ± 9.97 b	0.23 ± 0.15 a
Applying organic fertilizers	198.61 ± 41.72 a	17.54 ± 9.68 a	0.25 ± 0.16 a
Comprehensive improvement	206.72 ± 28.98 a	20.88 ± 10.58 a	0.33 ± 0.16 a
Non-agricultural land consolidation	183.58 ± 50.72 a	12.32 ± 11.87 c	0.22 ± 0.30 a

Note: The average value in the table is presented in the form of mean ± standard deviation. Different lowercase letters in the same column represent significant differences at the $p < 0.05$ level.

3.2.2. The Mechanism of Basic Physical and Chemical Properties of Soil on Fungal Community
Soil Physical Properties and Fungal Community

Soil particle size and soil moisture content are the key indicators of soil physical properties that affect the structure and diversity of the soil's microbial community, and have a strong influence on the structure of the soil's fungal community. Studies had shown that soil moisture content was the main environmental factor that affected the structure of fungal communities, and was significantly positively correlated with *Mortierella* and *Glomus*, especially in areas such as arid climates and coastal saline soils [46,47]. Soil particle size is a key factor that determines the storage and transportation of soil moisture and nutrients, and to a certain extent will affect the community structure and number of soil fungi [48]. Since soil particle size and soil moisture content are easily affected by farmland improvement projects, it is of certain significance to analyze the relationship between soil particle size, moisture content and the soil's fungal community structure in farmland consolidation areas.

Through redundancy analysis (db-RDA) based on Spearman's distance algorithm, the correlation coefficient r^2 of soil particle size, soil moisture content and the relative abundance of the fungal genus in the study area were 0.73 and 0.63, respectively, and the significance test value *p* was 0.001. According to the Spearman correlation measurement, the soil particle size and soil moisture content in the study area were significantly correlated with the relative abundance of the 8 fungal genera, but the impact of each farmland consolidation measure on the soil fungal community structure was quite different (Figure 9). In farmland consolidation areas where construction ditches were implemented, soil particle size was significantly positively correlated with the relative abundance of *Thanatephorus*, and significantly negatively correlated with the relative abundance of Penicillium. The soil moisture content in this type of farmland had a strong positive correlation with the relative abundance of fungi such as *Zopfiella* and *Acremonium*, but it was not significant. This was mainly because the construction of ditches could significantly improve the circulation of soil moisture in the farmland, which made the soil moisture in the area in a more balanced state, so there was no sudden increase in the correlation between fungi and soil moisture. However, if the soil particle size was too large, it would reduce the ability to adhere to humus and reduce the content of organic matter in the soil. As a heterotrophic aerobic fungus, *Penicillium* needed a lot of nutrients for growth and development. Therefore, there would be a strong negative correlation between soil particle size and *Penicillium*. At the same time, as the soil particle size increases, the soil moisture and nutrient cycle would be strengthened, which provided a better soil environment for the healthy growth of plants. The genus *Apothecium* is a pathogenic fungus that parasitizes plants, and it was easy to exchange nutrients with the host to improve its survival ability. In farmland consolidation areas where land leveling was implemented, soil moisture content and soil particle size had a strong negative correlation with the relative abundance of most fungi, but there was a significant positive correlation with the relative abundance of *Acremonium*. This was due to the fact that the farmland soil that implemented the leveling of the land was subjected to a certain mechanical compaction, which caused the physical properties of the

soil to deteriorate, such as the soil water circulation and air permeability, resulting in a general downward trend in the relative abundance of most soil fungi. However, *Acremonium* usually had strong adaptability and could grow rapidly in a soil environment with sufficient moisture and strong air permeability. In the farmland soil where combined plots were implemented, only the relative abundance of *Gibberella* had a significant positive correlation with soil moisture content, while the relative abundance of other fungi had no significant correlation with soil moisture content and particle size. This might be because the combined land did not involve extensive mechanical engineering and had little impact on soil fungi. In farmland soil with organic fertilizer, only the relative abundance of *Schizothecium* was significantly positively correlated with soil moisture content and particle size. This was because the main influencing factor of the fungal community structure in this area was soil nutrients, so the influence of soil physical properties was not great. At the same time, soil with sufficient water and a large particle size would accelerate the water cycle, which would lead to nutrient loss to a certain extent, which would reduce the relative abundance of some fungi. For farmland under comprehensive improvement, the interpretation of soil moisture content and particle size was the highest among all groups. It could be seen that after the implementation of comprehensive improvement, the physical properties of the soil would be greatly changed, and it would also cause a greater impact on the structure of the soil fungus community. In addition, the soil moisture content and particle size in the non-agricultural land consolidation area were significantly negatively correlated with the relative abundance of five fungi, including *Fusarium*, *Gibberella* and *Humicola*. This was mainly due to the limited drainage and nutrient supply in non-agricultural land consolidation areas, which restricted the growth and development of fungal communities.
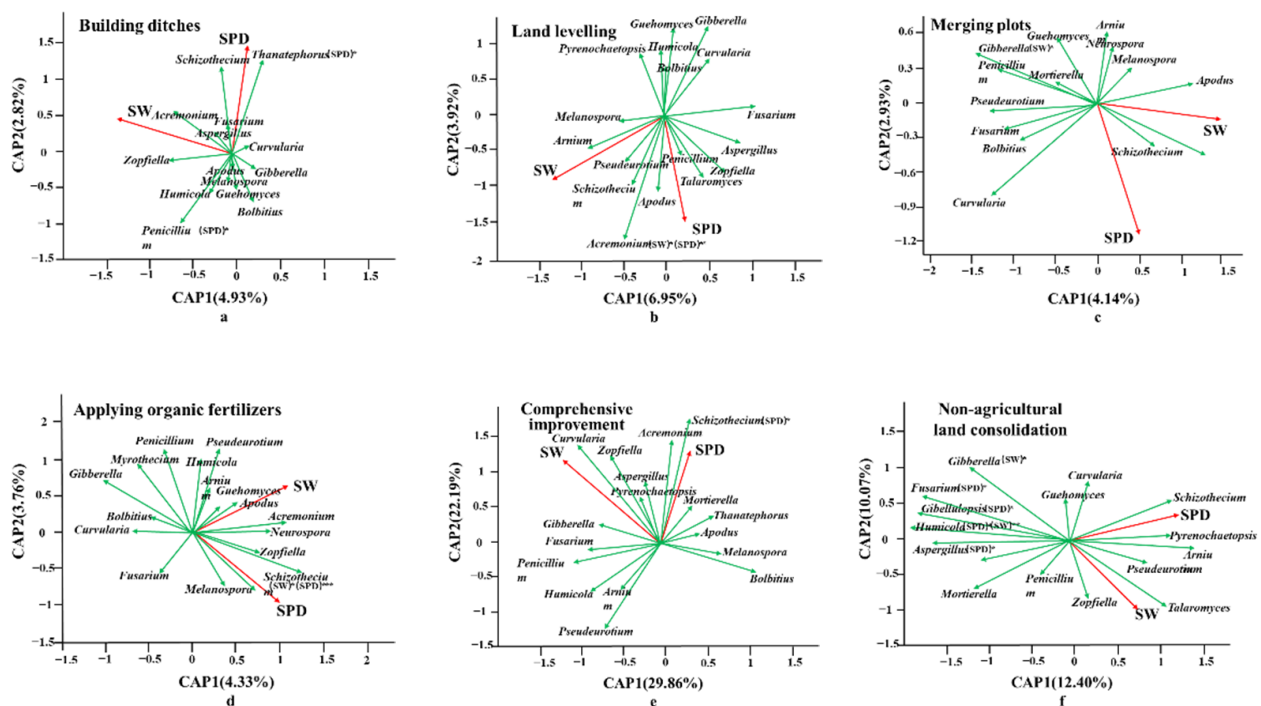

Figure 9. Redundancy analysis among different groups of soil particle size, moisture content and fungal community structure. (Note: The red arrow indicates the physical properties of the soil, and the length of the red arrow can indicate the degree of influence on the fungus; the green arrow indicates the fungal genus, and the projection is made from the green arrow to the red arrow. The distance between the projection point and the origin represents the effect of the physical properties of the soil. The degree of influence of fungal abundance, whether the directions of the two arrows are consistent, represents a positive and negative correlation. *, **, *** indicate significant correlation at the levels of 0.05, 0.01, and 0.001.)

Soil Chemical Properties and Fungal Communities

The soil fungal relative abundance, quantity and distribution are significantly affected by the basic chemical properties of the soil, including soil pH, organic matter (SOM), available phosphorus (AP), available potassium (AK) and total nitrogen (TN) [49]. Studies have shown that in a weakly alkaline soil environment, pH was significantly positively correlated with soil fungal diversity, and the *Chlorophyllum* was significantly positively correlated with pH at the 0.05 level. Experiments had shown that the application of organic fertilizer could significantly increase the relative abundance of *Blastocladiomycota*, *Chytridiomycota*, and *Zygomycota*, while the relative abundance of Basidiomycota showed a significantly reduced trend [50]. In wetland soil, organic matter content was the main environmental factor affecting the fungal community structure, especially the relative abundance of *Basidiomycota* [51]. In addition, many studies had also shown that soil AP, AK and TN were key factors affecting the structure and diversity of fungal communities [52]. The chemical properties of farmland soil were prone to significant changes under human interference, which would directly or indirectly affect the soil fungal community structure. Therefore, the research on how to change the soil chemical properties to affect the soil fungal community structure had a certain practical significance.

Through Mantel Test analysis, the soil chemical properties in the study area were significantly related to the fungal community structure ($r^2 = 0.13$, $p < 0.05$), especially the organic matter, pH, total nitrogen and other indicators. It could be seen that the chemical properties of the farmland soil in the study area had a strong influence on the soil fungal community structure. However, through Spearman's correlation analysis of different farmland improvement groups, it was found that the effects of chemical indicators on the relative abundance of fungi were quite different between different groups (Figure 10). In farmland consolidation areas where construction ditches were implemented, the relative abundance of *Apodus*, *Gibberella*, and *Talaromyces* was significantly positively correlated to soil nutrient elements such as SOM, AP, AK, and TN. However, eight fungi, including *Apodus*, *Bolbitius* and *Talaromyces*, had a strong negative correlation with soil pH. This was mainly because the construction of ditches could promote the circulation of soil moisture and nutrients, and provided an excellent growth environment for the widely distributed and reproductive strains of *Gibberella* and *Talaromyces*. At the same time, accelerating the circulation of soil water also helped to adjust the pH of the soil to gradually become neutral. This also caused the fungi adapted to the neutral soil environment to react more strongly to subtle pH changes, so the relative abundance of soil fungi in this area had a strong correlation with pH. In farmland with leveled land and combined plots, the relative abundance of fungi was significantly negatively correlated with soil chemical properties. Only the relative abundance of *Aspergillus*, *Curvularia*, and *Acremonium* was significantly positively correlated with soil chemical properties. This was because the soil was subjected to mechanical compaction and other effects, resulting in a decrease in soil aeration performance, water circulation and nutrient circulation. Although the farmland consolidation area had high soil nutrients and a suitable acid-base environment, it was still not conducive to the growth and reproduction of some fungi [52]. In farmland soil where organic fertilizer was applied, the relative abundance of 11 species of fungi was significantly related to the chemical properties of the soil. This was mainly because the application of organic fertilizer significantly increased the soil organic matter and other nutrients, and promoted the growth and reproduction of fungi. However, there were also situations in which dominant strains squeeze other inferior strains, resulting in a significant positive or negative correlation between more fungi in this area and soil chemical properties. Compared with the comprehensive improvement area, the relative abundance of soil fungi in non-agricultural land consolidation areas had a higher correlation with soil chemical properties. This was mainly due to the lower soil nutrient content and greater pH fluctuations in the non-agricultural land consolidation area, and the soil fungal community structure was more susceptible to influence.
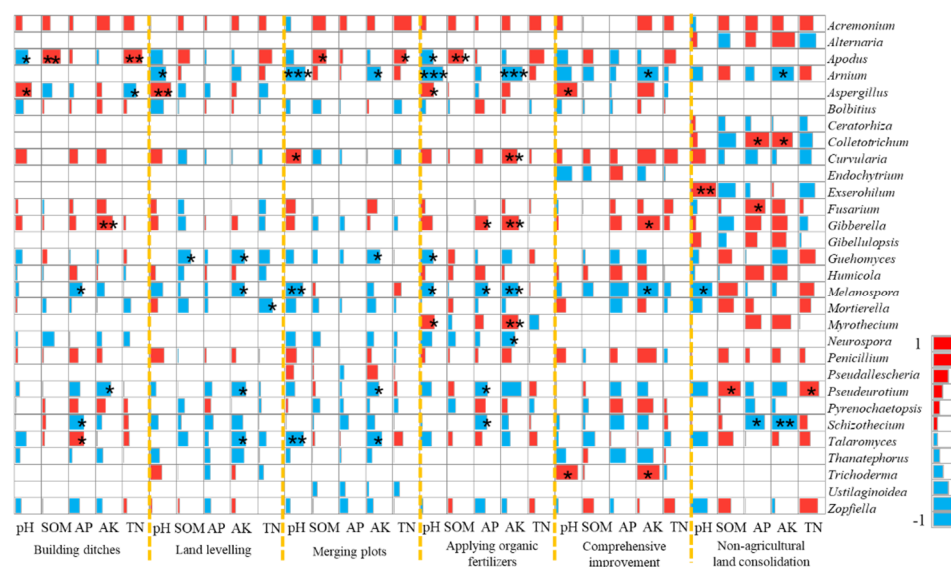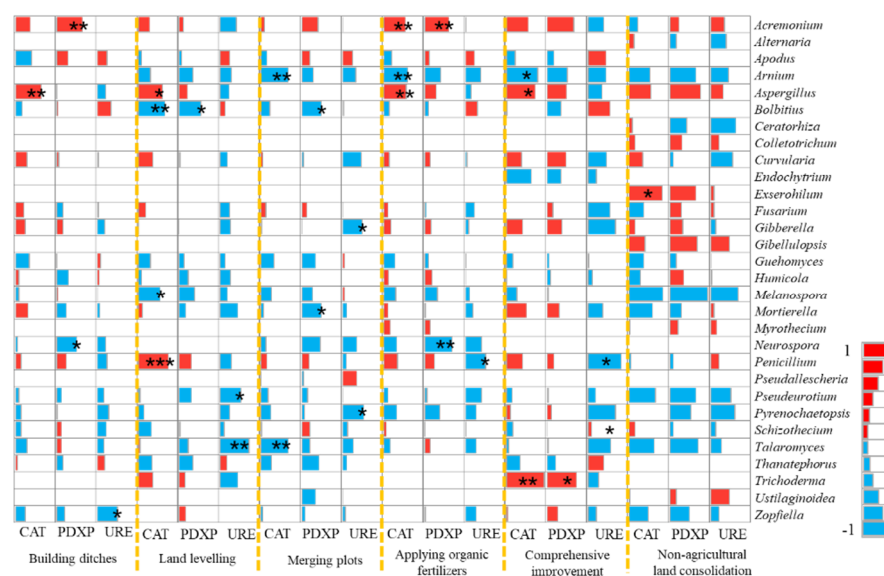
Figure 10. Correlation between soil chemical properties and fungal community structure in different groups. (Note: In the figure, warm colors indicate positive correlation, and cool colors indicate negative correlation. *, **, *** indicate significant correlation at the levels of 0.05, 0.01, and 0.001, respectively).

Soil Enzyme Activity and Fungal Community

Soil fungi were important members of the soil micro-ecological environment, and participate in soil biochemical processes such as releasing soil enzymes, degrading organic matter, and regulating nutrient cycling. At the same time, soil enzymes were closely related to soil fungi and were key biological indicators to measure soil's environmental dynamics and fertility levels [53]. Soil urease (URE), phosphatase (PDXP) and catalase (CAT) activities in the soil could effectively characterize the nutrient elements such as carbon and nitrogen in the soil, and the status of the soil biological activity [54]. Previous studies mainly focused on the relationship between soil enzyme activity and the number and diversity of fungi, and seldom involved detailed fungal species [55,56]. Therefore, it had certain research significance to analyze the correlation between soil enzyme activity and the fungal genus structure in different soil environments in farmland consolidation areas.

A total of 16 fungal genera in farmland soil samples in the study area were significantly correlated with soil enzyme activity indicators such as URE, PDXP and CAT (Figure 11). In the farmland where construction ditches and land leveling were implemented, two fungi were significantly positively correlated with soil enzyme activities, while the farmland soil fungi and soil enzyme activities were mainly negatively correlated with the combined plots. This was mainly because the construction of ditches and land leveling could improve the supply of soil moisture and nutrients, creating favorable conditions for the growth of soil fungi and the improvement of soil enzyme activities. However, most of the farmland that had been merged had not effectively improved the basic agricultural facilities, and so had no beneficial impact on the soil's fertility and micro-ecological environment. At the same time, compared with non-agricultural land consolidation areas, soil fungi and enzyme activities had a stronger positive correlation between the areas where organic fertilizers were applied and comprehensive improvement were carried out. In particular, *Acremonium*, *Aspergillus*, and *Trichoderma* were significantly positively correlated with catalase (CAT) and phosphatase (PDXP). This was mainly because the farmland soil with organic fertilizer and comprehensive improvements had higher nutrients. Fungi could obtain these nutrients to achieve rapid reproduction and secrete more soil enzymes. In the soil of non-agricultural land consolidation areas, the relative abundance of most fungi was negatively correlated with soil enzymes. This was also because there were fewer nutrients available for soil enzyme decomposition in this grouping, and the overall ecological environment of the soil

was poor, resulting in that the soil fungal community structure could not be effectively improved. These results were highly consistent with previous studies [54,55].

Figure 11. Correlation between soil enzyme activity and fungal community structure in different groups. (Note: In the figure, warm colors indicate positive correlation, and cool colors indicate negative correlation. *, **, *** indicate significant correlation at the levels of 0.05, 0.01, and 0.001, respectively).

3.3. Farmland Consolidation Regulates Heavy Metal Soil Content and Its Mechanism of Action on Fungi

3.3.1. Effects of Farmland Consolidation on Heavy Metal Soil Content

Heavy Metal Content of Farmland Soil

The levels of heavy metals in farmland soils of different groups in the study area were shown in Table 5. It could be seen from Table 5 that the average content of three heavy metals in the eight types of heavy metals tested in the farmland soil of the study area was greater than the background value of the soil. Among them, the average contents of Cu, Cd, Pb, Cr, As, Hg, Ni, and Zn were 45.17, 2.5, 41.94, 223.79, 5.29, 0.55, 56.77, 123.27 mg/kg, respectively. They were 0.45, 8.33, 0.14, 0.75, 0.21, 1.1, 1.14, and 0.49 times the national standard, and the rate of exceeding the standard was 38%. This showed that there was a certain degree of enrichment of heavy metals in farmland soils in this study area.

In addition, the order of the coefficient of variation of the eight heavy metals in the study area was: As > Hg > Pb > Cd > Cu > Zn > Ni > Cr. Among them, the coefficients of the variation of As, Hg, Pb, Cd, Cu and Zn were all greater than 20%, belonging to moderate intensity variation, indicating that these heavy metals were significantly affected by external interference. The specific manifestation was that the content of heavy metals in the soil varies greatly in space, which was mainly attributed to the influence of human disturbance factors such as farming methods, fertilizer application, management measures, and pollutant emissions. The coefficient of variation of Cr and Ni was relatively small, indicating that the spatial distribution of these two heavy metal elements was relatively uniform, and there might be a certain degree of homology [57]. As an artificial measure that strongly disturbs the soil environment, farmland consolidation was an important factor affecting the spatial distribution of heavy metal soil content. There was a big difference in the content of heavy metals between the areas where different agricultural land consolidation measures were implemented and the non-agricultural land consolidation areas, and the highest value of the average content of various heavy metals was in the non-agricultural land consolidation area. The content of heavy metals in farmland where the construction of ditches, land leveling, combined plots, application of organic fertilizers, and comprehensive improvement was implemented was lower than that of non-agricultural land consolidation

areas. This was mainly because the construction of ditches could significantly increase the transfer effect of soil moisture on heavy metals, and the application of organic fertilizers could provide nutrients for the growth and reproduction of microorganisms to play the function of transferring and absorbing related heavy metals, which was consistent with the existing research results [58].

Table 5. Soil (0–15 cm) heavy metal content in the study area. Unit: mg/kg.

	Cu	Cd	Pb	Cr	As	Hg	Ni	Zn
Building ditches	45.95 ± 12.74	2.28 ± 0.23	43.39 ± 13.23	214.65 ± 11.67	4.66 ± 3.83	0.57 ± 0.28	53.15 ± 5.60	125.75 ± 26.70
Land levelling	39.44 ± 7.92	2.23 ± 0.27	37.54 ± 12.81	214.61 ± 11.34	4.21 ± 3.51	0.45 ± 0.15	54.06 ± 7.63	110.47 ± 18.39
Merging plots	41.72 ± 8.83	2.27 ± 0.33	37.62 ± 12.06	212.64 ± 11.22	4.99 ± 3.91	0.53 ± 0.25	54.55 ± 7.44	112.51 ± 21.58
Applying organic fertilizers	46.55 ± 12.25	2.27 ± 0.31	42.98 ± 10.38	216.45 ± 11.12	3.89 ± 2.90	0.53 ± 0.21	55.00 ± 6.86	128.36 ± 24.72
Comprehensive improvement	39.90 ± 9.17	2.28 ± 0.13	40.29 ± 14.98	208.70 ± 6.59	2.93 ± 3.94	0.47 ± 0.14	51.10 ± 6.93	117.82 ± 20.45
Non-agricultural land consolidation	48.97 ± 6.71	3.30 ± 0.55	46.83 ± 8.81	252.96 ± 11.05	7.88 ± 5.29	0.65 ± 0.37	64 ± 10.85	136.82 ± 21.17
Maximum	84	3.98	77.5	268.8	14.4	1.38	80.4	198
Minimum	25	1.57	20	195	0.81	0.1	39	70.6
Average value	45.17	2.5	41.94	223.79	5.29	0.55	56.77	123.27
Variation coefficient (%)	22.38	22.86	26.4	8.72	77.91	49.99	15.96	20.7
National standard	100	0.3	300	300	25	0.5	50	250

Note: The content of heavy metals in the table is presented in the form of average ± standard deviation.

Heavy Metal Pollution Level of Farmland Soil

According to the calculation result of the geological accumulation index (GI) (Figure 12), the pollution degree of the heavy metals in the study area was ranked as follows: Cd > Cr > Hg > Ni > Cu > Zn > Pb > As. Among them, Cd was the heavy metal with the highest geological accumulation index and the most polluted heavy metal in the study area. The geological accumulation index of the three heavy metals Pb, As and Zn was mostly negative, indicating that the soil in the study area was not contaminated by them [59]. At the same time, there were big differences in the geological accumulation level of heavy metals among different groups. In the study area, the cumulative geological index of heavy metals in the farmland under comprehensive improvement was generally low, while the geological cumulative index of heavy metals in the non-agricultural land consolidation area was the highest.
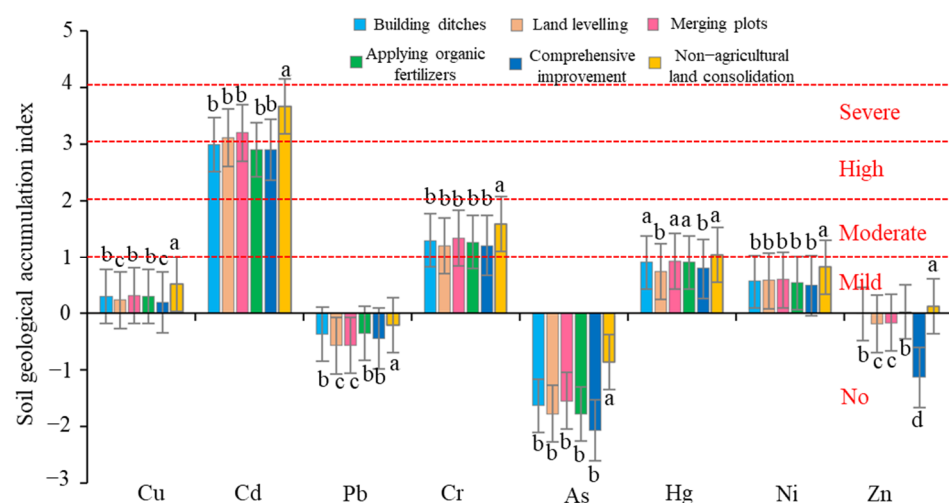
Figure 12. Evaluation results of soil geoaccumulation index in the study area. Note: Different lowercase letters represent significant differences at the $p < 0.05$ level.

The comprehensive pollution level of heavy metals in farmland soil could be determined by measuring the Nemerow comprehensive index (NI). The average range of the soil Nemerow comprehensive index for each group in the study area was 0.64 to 3.68.

According to the heavy metal soil pollution index classification standard [28], when the Nemeiro composite index was less than 1, the soil had low pollution; when the Nemeiro composite index was 1 to 2, the soil was lightly polluted; when the Nemeiro composite index was 2 to 3, the soil was moderately polluted, which would pose a toxic threat to rice; when the Nemeiro composite index was 3 to 4, the soil was heavily polluted, which would seriously affect the growth and development of crops. According to the calculation results of Nemeiro composite index, it could be seen that the farmland soil in the non-agricultural land consolidation area was at a heavy metal pollution level. The farmland soil of the combined plot was at a moderate level of heavy metal pollution, while the farmland soil in other farmland remediation areas was at a light pollution level (Figure 13). This was because the implementation of construction ditches could promote the soil water cycle to improve the effective transfer of heavy metals, and the application of organic fertilizer could increase the abundance of heavy metal-tolerant fungi to adsorb, migrate and detoxify related heavy metals. All of these could effectively reduce the level of heavy metal pollution in farmland consolidation areas and were consistent with existing research results [58]. At the same time, this also explained why the farmland soil that implemented comprehensive farmland improvement had the lowest level of heavy metal pollution.
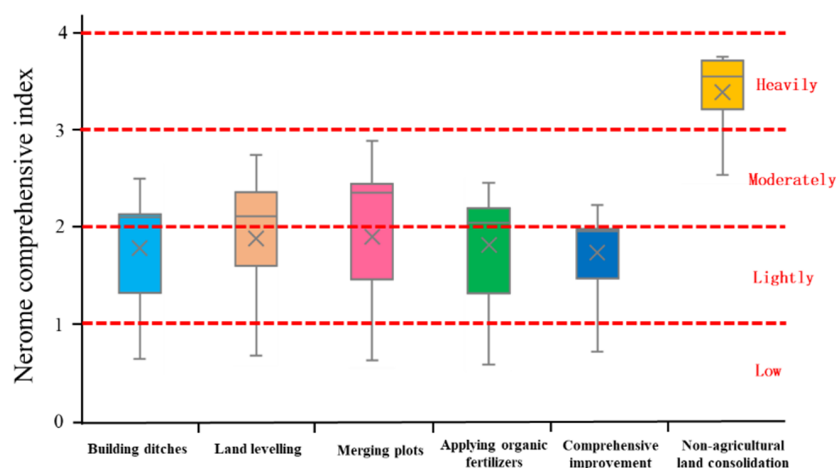

Figure 13. Evaluation results of the Merrow comprehensive pollution index in the soil of the study area.

Through the above calculations, analyses and field investigations, it could be seen that the reasons for the large differences in the level of heavy metal pollution in farmland soils in the study area were: (1) In recent years, the area where the study area was located had continuously strengthened the development and utilization of industrial areas, resulting in continuous industrial pollutants. It flowed into the surrounding farmland, causing serious soil pollution by heavy metals. (2) Many oily pollutants, such as fertilizer bags, pesticide bottles, and herbicides, which were likely to cause cadmium (Cd) pollution, were scattered in the farmland, but had not been effectively removed. (3) Pollutants caused by fertilizer application, poultry breeding, and domestic garbage in the study area were also sources of heavy metal pollution in the soil [60]. (4) Measures such as building ditches and applying organic fertilizers in farmland consolidation areas could accelerate water circulation to transfer heavy metals, and provide nutrients for fungi with heavy metal repair functions to achieve the adsorption and detoxification of heavy metals. This had effectively reduced the level of heavy metal pollution in farmland [61].

3.3.2. The Mechanism of Heavy Metal Soil Pollution on Fungal Communities
Fungal Communities at Low Pollution Levels

A total of 10 samples in the study area belonged to the low level of heavy metal pollution, all of which were located in the farmland consolidation area. According to CCA analysis, among these 10 samples, heavy metals Cd ($r^2 = 0.26$, $p = 0.031$), Hg ($r^2 = 0.28$,

p = 0.032), and Ni (r² = 0.50, *p* = 0.010) were significantly related to fungal community structures. According to the Spearman correlation analysis, the content of heavy metals was significantly related to two fungal phyla and seven fungal genera (*p* < 0.05) (Figure 14).
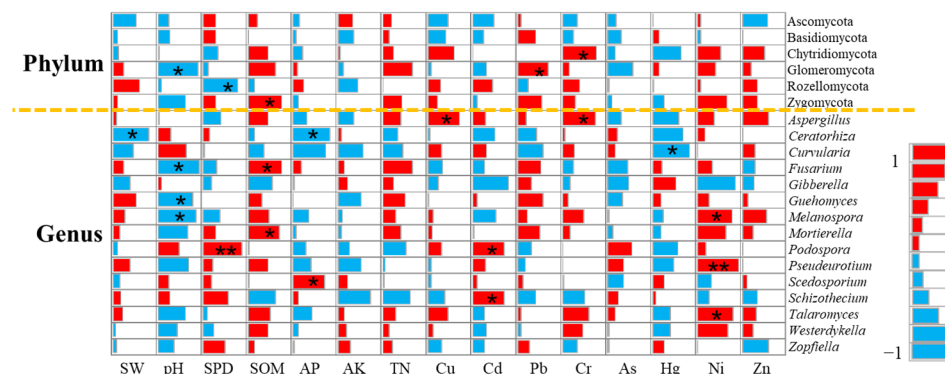
Figure 14. Correlation between soil environmental factors and fungal community structure at low levels of heavy metal pollution. (Note: In the figure, warm colors indicate positive correlation, and cool colors indicate negative correlation. *, ** ndicate significant correlation at the levels of 0.05, 0.01, and 0.001, respectively).

At the fungal phyla level, the relative abundance of the soil fungi Glomeromycota, *Rozellomycota*, and *Zygomycota* in farmland with low heavy metal soil pollution levels was significantly related to the soil's physical and chemical properties, such as pH, SPD, and SOM. The relative abundances of *Chytridiomycota* and *Glomeromycota* in the soil in this area were significantly positively correlated with the content of heavy metals such as Pb and Cr, respectively. It could be seen that these two kinds of fungi had strong adaptability under the low pollution level of heavy metals, and could absorb and transfer the two heavy metals of Pb and Cr to a certain extent.

At the level of the fungi genus, there were 7 species of fungi that were significantly related to the basic physical and chemical properties of soil, such as SW, pH, SPD, SOM, and AP. The heavy metals Cu, Cd, Cr, Hg, Ni were the main influencing factors affecting the fungal genus structure, and the relative abundance of *Aspergillus* was significantly positively correlated with the content of Cu and Cr. *Podospora* and *Schizothecium* were significantly positively correlated with Cd, and *Melanospora*, *Pseudeurotium* and *Talaromyces* were significantly positively correlated with Ni. This showed that these fungi had a certain tolerance to heavy metals such as Cu, Cd, Cr, and Ni in the low pollution level of heavy metals, and had a strong function of heavy metal degradation, which had a great effect on improving the soil environment.

Fungal Communities at Light Pollution Levels

A total of 14 samples in the study area belonged to the light pollution level of heavy metals. According to CCA analysis, among the 14 samples, heavy metals Cd (r² = 0.31, *p* = 0.015), Hg (r² = 0.16, *p* = 0.034), and Ni (r² = 0.48, *p* = 0.005) were significantly related to the fungal community structure. According to the Spearman correlation analysis, the content of heavy metals was significantly related to 1 fungal phylum and 7 fungal genera (*p* < 0.05) (Figure 15).

At the phylum level, the relative abundance of soil fungi in farmland at this pollution level was significantly related to the physical and chemical properties of soil, such as SW and pH, especially for the phyla Ascomycota and Glomeromycota. Among the heavy metals, Cu and Cd had a strong influence on the fungal community structure, which was significantly related to the relative abundance of the fungus Zygomycota. In addition, the content of Cu was positively correlated with the 6 kinds of mycota, while the content of Cd was negatively correlated with the 6 kinds of mycota. It could be seen that under the environment of mild heavy metal pollution, heavy metal Cd had a strong inhibitory effect

on the growth of fungi, but fungi had a strong tolerance to Cu, and could play a certain role in adsorption and degradation.
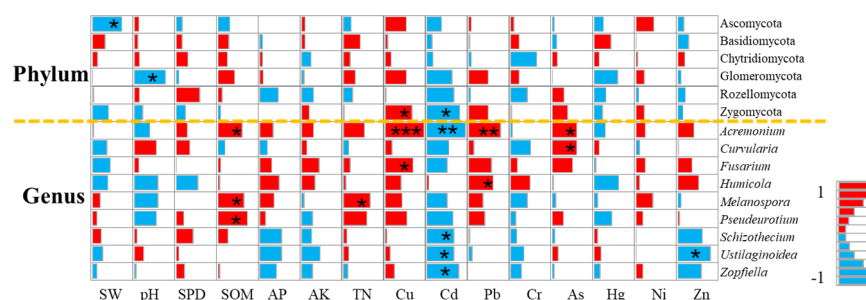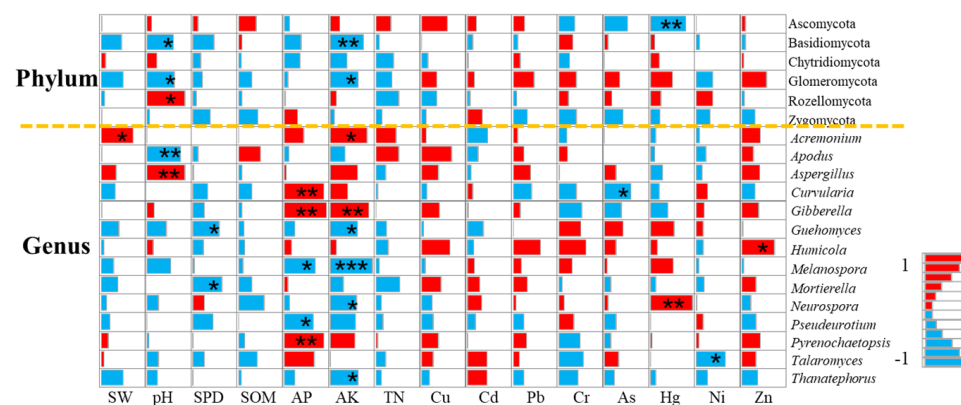

Figure 15. Correlation between soil environmental factors and fungal community structure at lightly polluted levels of heavy metals. (Note: In the figure, warm colors indicate positive correlation, and cool colors indicate negative correlation. *, **, *** indicate significant correlation at the levels of 0.05, 0.01, and 0.001, respectively).

At the genus level, the SOM, TN and other indicators in the basic physical and chemical properties of soil were important influencing factors affecting the structure of the fungal community, and had a significant positive impact on the relative abundance of *Acremonium*, *Melanospora*, *Pseudeurotium*, and other fungal genera. In addition, Cu, Cd, Pb, As, and Zn were the main heavy metal elements that affected the fungal genus structure. Among them, the relative abundances of *Acremonium* and *Fusarium* were significantly positively correlated with Cu content, *Acremonium* and *Humicola* were significantly positively correlated with Pb, and *Acremonium* and *Curvularia* were significantly positively correlated with As. This showed that these fungi had a certain tolerance to heavy metals such as Cu, Pb, As and other heavy metals in the light pollution level of heavy metals, and had a strong function of heavy metal degradation, which had a great effect on improving the soil environment.

Fungal Communities at Moderate Pollution Levels

A total of 15 samples in the study area belonged to the moderate pollution level of heavy metals. According to CCA analysis, among the 14 samples, the heavy metals Cu ($r^2 = 0.59$, $p = 0.043$), Cr ($r^2 = 0.33$, $p = 0.015$), and Hg ($r^2 = 0.23$, $p = 0.026$) were significantly related to the fungal community structure. According to Spearman's correlation analysis, the content of heavy metals was significantly related to 1 fungal phylum and 4 fungal genera ($p < 0.05$) (Figure 16).
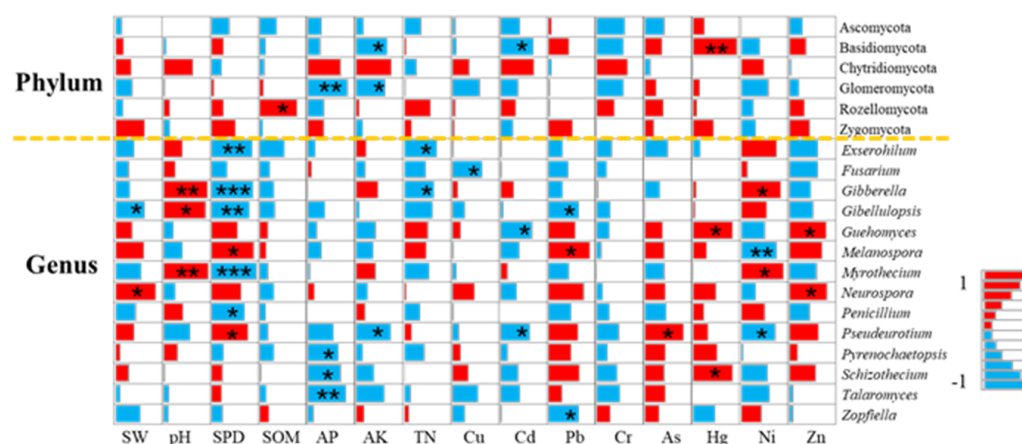

Figure 16. Correlation between soil environmental factors and fungal community structure at moderate levels of heavy metal pollution. (Note: In the figure, warm colors indicate positive correlation, and cool colors indicate negative correlation. *, **, *** indicate significant correlation at the levels of 0.05, 0.01, and 0.001, respectively).

At the phylum level, the relative abundance of fungi such as Basidiomycota and Glomeromycota in farmland soils with moderate pollution levels was significantly negatively correlated with the basic physical and chemical properties of soil, such as pH and AK. Among the heavy metal elements, only the Hg element content had a significant negative correlation with the relative abundance of Ascomycota. It could be seen that the growth of fungal communities was restricted to a greater extent in environments with moderately polluted heavy metals.

At the genus level, the pH, SPD, AP, AK and other indicators in the basic physical and chemical properties of soil were important influencing factors on the fungal community structure, and had a significant impact on the relative abundance of *Acremonium*, *Gibberella*, *Melanospora*. The influence of heavy metals on the relative abundance of fungal genera was limited. Only Hg and Zn were significantly positively correlated with *Neurospora*, *Humicola* and other fungal genera, respectively. This also showed that under this pollution level, the basic physical and chemical properties of the soil had a greater impact on the community structure of fungi, and some fungi still had a strong degrading effect on heavy metals such as Hg and Zn.

Fungal Communities under Heavy Pollution Levels

A total of 11 sample points in the study area were in areas heavily polluted by heavy metals. According to CCA analysis, among the 11 samples, heavy metals Cd ($r^2 = 0.46$, $p = 0.014$), Pb ($r^2 = 0.37$, $p = 0.020$), and Ni ($r^2 = 0.81$, $p = 0.001$) were significantly related to fungal community structure. According to the Spearman correlation analysis, the content of heavy metals was significantly related to 1 fungal phylum and 10 fungal genera ($p < 0.05$) (Figure 17).

Figure 17. Correlation between soil environmental factors and fungal community structure at the level of heavy metal pollution. (Note: In the figure, warm colors indicate positive correlation, and cool colors indicate negative correlation. *, **, *** indicate significant correlation at the levels of 0.05, 0.01, and 0.001, respectively).

At the phylum level, the relative abundance of fungi such as Basidiomycota, Glomeromycota, Rozellomycota in farmland soils at the heavily polluted level was significantly correlated with soil nutrients such as SOM, AP, and AK. Both Cd and Hg had a significant effect on the relative abundance of Basidiomycota, and Hg had a significant positive correlation with Basidiomycota at the level of 0.001. In addition, there was a strong positive correlation between Chytridiomycota, Rozellomycota, Zygomycota, and many kinds of heavy metals. This showed that there were still a certain number of heavy metal-tolerant fungal phyla in heavy metal pollution environments, which played an important role in stabilizing the quality of the soil environment.

At the genus level, the basic physical and chemical properties of soil such as SW, pH, SPD, AP, and TN had a greater impact on the fungal community structure, especially

with the relative abundance of *Gibberella*, *Gibellulopsis* and *Myrothecium*. At the same time, the relative abundance of most fungal genera had a strong negative correlation with soil nutrient indexes. This might be due to the fact that in this environment, the growth of fungi was extremely sensitive to the basic physical and chemical properties of the soil under the influence of heavy metal stress. The content of heavy metals such as Cu, Cd, Pb, As, Hg, Ni, and Zn had a great influence on the genus structure of the soil fungi. Among them, Pb content was significantly positively correlated with the relative abundance of Melanospora, As was significantly positively correlated with *Pseudeurotium*, Hg was significantly positively correlated with *Guehomyces* and *Schizothecium*, Ni was significantly positively correlated with *Gibberella* and *Myrothecium*, and Zn was significantly positively correlated with *Guehomyces* and *Neurospora*. It could be seen that under heavy metal pollution levels, soil fungi were extremely sensitive to changes in the soil environment, and heavy metal-tolerant fungi would consume more soil nutrients in the process of absorbing and detoxifying heavy metals, resulting in a significant negative correlation between soil nutrients and soil fungi.

4. Conclusions

This paper used soil experimental analysis and high-throughput sequencing technology of gene amplicons to measure the basic physical and chemical properties of soil, heavy metal content, and microbial properties. The basic physical and chemical properties of soil and heavy metal content were used as an intermediary to study the impact mechanism of farmland consolidation on microorganisms. This study explored the effects of different farmland consolidation measures on the soil's micro-ecological environment and drew the following main conclusions:

(1) Farmland improvement had a significant impact on soil microbial properties, which were mainly manifested in changes in soil microbial biomass, microbial diversity and community structure. The soil microbial biomass of carbon and nitrogen in farmland consolidation areas were significantly higher than those in non-agricultural land consolidation areas, and the microbial biomass of phosphorus in soil samples from most farmland remediation areas was significantly higher than that in non-agricultural land consolidation areas. In the study area, the soil fungal community richness indexes, Sobs and Chao, of cultivated land that had been renovated on farmland, were significantly higher than those in the non-agricultural land consolidation area at the $p < 0.05$ level. The soil fungal community diversity index, Shannon, in the areas where comprehensive improvement was implemented, was significantly lower than that in other areas, while the soil fungal community diversity index, Invsimpson, in the non-agricultural land consolidation area, was significantly higher than that in the farmland consolidation area. Regarding the microbial community structure, farmland consolidation could increase the relative abundance of the three dominant fungal phyla, but it also had a negative impact on the relative abundance of the three dominant fungal phyla. The pathogenic fungi Gibberella, Fusarium, and Curvularia had the highest relative abundance in the non-agricultural land consolidation area, but the lowest relative abundance in the comprehensive consolidation area.

(2) Farmland consolidation had an indirect impact on soil microorganisms by adjusting the basic physical and chemical properties of the soil. Studies had shown that there were great differences in the effects of different farmland consolidation measures on the relative abundance of soil fungal communities. For the fungal community, the soil particle size and the relative abundance of fungal genera, such as *Thanatephorus*, *Acremonium*, *Schizothecium*, under different farmland consolidation measures were significantly positively correlated, and have a significant negative correlation with the relative abundance of *Penicillium*. The soil moisture content was significantly positively correlated with the relative abundance of *Schizothecium* and *Gibberella*. Soil pH was significantly related to the relative abundance of *Apodus* and *Talaromyces*. SOM, AP, AK, TN and other nutrients had a significant positive correlation with the relative abundance of Gibberella and Talaromyces. Soil enzyme activity was significantly correlated with a variety of fungi. This was mainly the adaptive

adjustment of soil fungal communities in farmland consolidation areas affected by changes in soil water cycle, soil adhesion, pH, and nutrient content. In the non-agricultural land consolidation area, the soil moisture content and particle size were significantly negatively correlated with the relative abundance of five fungi including *Fusarium*, *Gibberella* and *Humicola*. Nutrients such as SOM, AP AK, and TN had a higher correlation with the relative abundance of fungi, while the soil enzyme activity had a negative correlation with the relative abundance of most fungi. This was mainly due to the limited drainage and nutrient supply in the non-agricultural land consolidation area, which restricted the growth and development of the fungal community and caused the soil fungal community structure to not be effectively improved.

(3) The impact of heavy metals on the fungal community structure varies greatly under different levels of heavy metal pollution. Cultivated lands with low pollution levels were all located in farmland consolidation areas. The fungal phylums Chytridiomycota and Glomeromycota had certain absorption and transfer functions for Pb and Cr, respectively. In addition, there were six fungal genera that were significantly positively correlated with heavy metals such as Cu, Cd, Cr, and Ni. Most of the soil samples at a lightly polluted level were located in farmland consolidation areas. In this area, there were six fungal phyla that were positively correlated with the content of Cu. Fungi such as *Acremonium*, *Fusarium*, *Humicola*, and *Curvularia* had a certain tolerance to heavy metals such as Cu, Pb and As, and had strong heavy metal degradation functions. Among the soil samples with moderate pollution levels, 13 samples were located in the farmland consolidation area, and only the Hg content was significantly negatively correlated with the relative abundance of the Ascomycota. The fungi genus *Neurospora* and *Humicola* were significantly positively correlated with Hg and Zn content, respectively.

With the continuous increase of heavy metal pollution, the number of heavy metal-tolerant fungi in the soil generally increased first and then decreased. This was mainly due to the increase in pollution level, which stimulated the vitality of heavy metal-tolerant fungi and showed better heavy metal adsorption and detoxification functions. However, as the pollution continues to intensify, some fungal species were eliminated and the dominant fungi were left. Soil fungal communities were more sensitive to changes in the content of heavy metals, and the number of heavy metal-tolerant fungi changed drastically with the increase of pollution. When the pollution level was at the highest level, 7 species of fungi showed a strong tolerance to heavy metals and also consumed a lot of soil nutrients. These strains, with strong heavy metal absorption and detoxification functions in heavy metal pollution environments, could be used as effective bioremediation methods to improve the soil environment. They should be added to China's cultivated land quality evaluation system to serve the cultivated land quality improvement project in farmland consolidation.

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