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Short-Term Response of Soil Microbial Community to Field Conversion from Dryland to Paddy under the Land Consolidation Process in North China

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Abstract: Land consolidation of dryland-to-paddy conversion for improving tillage conditions and grain production capacity is widely implemented throughout the world. The conversion affects soil ecological stability, especially the most active soil microorganisms. However, the impacts of the dryland-to-paddy conversion has paid little attention in recent decades. In this study, a pot experiment was used to explore the responses of the microbial community and their interactions with soil properties after rice in the first season (five months). The results indicated that a significant decrease in the topsoil pH, organic matter content, nitrate nitrogen, and ammonical nitrogen, and an increase in soil electrical conductivity (EC) was observed ($p < 0.05$) after the dryland-to-paddy conversion. The richness and diversity of bacteria and fungi decreased in the short term. The composition of the soil microbial community and the soil microbial dominant bacteria had considerably changed after the conversion. *Actinobacteria*, *Firmicutes*, and *Olpidiomycota* were found to be highly sensitive to the dryland-to-paddy conversion. The soil microbial community structure had extremely significant positive correlations with soil pH, EC, organic matter, nitrate nitrogen, and ammonical nitrogen ($p < 0.05$). Microorganisms are the most important component of soil nutrient cycling. Converting a large area of dryland to paddy may lead to an imbalance in the soil carbonitride cycle and should be further examined in North China.

Keywords: land consolidation; soil microorganisms; high-throughput sequencing; bacterial diversity; fungal diversity

1. Introduction

The soil carbon pool is the world's largest carbon pool in the terrestrial ecosystem [1–3]. Even a slight change in the soil can lead to huge changes in the global carbon cycle, and even create an imbalance [4,5]. Changes in the soil carbon pool are usually attributed to changes in land use, which are closely associated with human socioeconomic activities [6–8]. To cope with the rapid loss of agricultural land and food security issues, since 2000, the Chinese government has invested \$436 billion in large-scale land consolidation that has covered 30,000 hectares cumulatively, with North China accounting for approximately 60% of the land consolidation projects [9,10]. This large-scale land consolidation project has significantly impacted the agricultural ecological environment, such as the carbon cycle, coordination of soil and water utilization, and agricultural ecological services [11–14].

The most active components of the soil environment are the microorganisms that are critical for the nutrient cycling in soil [15–18]. Previous studies have shown that 58–90% of the carbon flux in the decomposition of soil organic matter (SOM) is produced by bacteria and fungi [19,20]. Microorganisms alter the soil development process and greenhouse gas emissions by affecting the decomposition and accumulation of organic matter (OM) in the soil [20]. Soil conversion affects the physiochemical properties and microbial community of agricultural soil [15], as well as soil carbon cycling [21]. Paddy rice agriculture provides the staple grain for almost half of China's population and the rice yield is higher than that of other crops [22]. Because of the current policy of protecting cultivated land “to compensate excellent land for excellent land taken” and “to compensate paddy field for paddy field taken” by the Chinese government [23], some areas with superior hydrothermal conditions, such as sufficient rainfall, higher annual accumulated temperature, and flat terrain, are often converted from dryland to paddy during the land consolidation process [22].

However, dry land and paddy fields are two distinct land uses, and they have significantly different impacts on the soil environment and the microbial community [24]. By changing the community structure of the soil, different tillage methods may result in different changes in soil biological richness and evenness [11,13]. The land consolidation of dryland-to-paddy conversion affects the soil environmental factors and the quality of the soil matrix, which eventually affects the diversity and functioning of the soil microbial community [25–27]. The large-scale conversion of dryland to paddy would inevitably alter the local soil environment or even a large area of agricultural land, and affect the physicochemical properties and biological processes of the soil [11–13], thus directly or indirectly affecting the agroecosystem carbonitride cycle and the emissions of greenhouse gases such as CO₂, CH₄, and N₂O [17]. Dryland-to-paddy conversion would impact the ground surface radiation balance and ground surface temperature because of the difference in land cover, thus affecting the regional climate characteristics [22,28]. Yu et al. hypothesized that land consolidation is an important measure to implement for meeting human food demand by increasing cultivated areas; however, concomitantly, the other ecosystem services are weakened, such as landscape diversity, which may cause a series of ecological problems, such as biomass decreases, thereby threatening the biodiversity [29].

Since 2000, China has conducted large-scale land consolidations, with the initial goal of supplementing arable land and increasing food production capacity [30–34]. In 2008, the Chinese government demanded “a large-scale implementation of rural land consolidation” and emphasized on improving the quality of cultivated land [23]. After 2013, the ecological transformation of land consolidation became a national agenda [30–32]. To meet the increasing demands on rice production because of the growing population, paddy rice agriculture has been expanding to the northern regions of China [22]. The conversion of dryland to paddy through land consolidation has become a common phenomenon in North China. Although previous studies have evaluated various aspects, the socio-economic objective of land consolidation has been long overstated [33], and little attention has been paid to its potential negative impacts on the ecological environment. In this study, the change in soil microbial community after dryland-to-paddy conversion and its relationship with the soil environment were investigated through simulation experiments and high-throughput sequencing technology. These findings are expected to provide a basis for the formulation of policies related to land consolidation, intensive agriculture, and technology advancements for the dryland-to-paddy conversion implemented by the Chinese government.

2. Materials and Methods

2.1. Experimental Field and Design

The studied areas were the South Lake Campus of the China University of Mining and Technology, Xuzhou, Jiangsu, China (117°08'08" E and 34°12'31" N). The climate is warm temperate semi-humid monsoon with four distinct seasons every year and abundant sunlight. The annual average temperature

is 14 °C, the average annual precipitation is 841.2 mm, and the frost-free period is 209 days [34]. Xuzhou is a part of the North China Plain and is a traditional wheat-corn rotation area. Affected by factors like soil salinization, drought, and farmland fragmentation, Xuzhou faces high agricultural vulnerability [35]. Xuzhou has been vigorously promoting land consolidation by converting dryland to paddy to upgrade farmland water conservancy facilities and to improve the stability of agricultural production.

The dryland to paddy conversion was simulated in the experimental fields of the Land Science Research Center, located in the South Lake Campus of China University of Mining and Technology (Xuzhou, Jiangsu, China), from June to November 2018. These fields had been in use for wheat-corn planting for the previous five years. It belonged to the agricultural ecosystem with wheat-corn planting. The soil composition was cinnamon fluvo-aquic with a sand-silt-clay ratio of 15.12:22.34:9.15:53.39 [34]. We set up two groups of pot experiments: one group planting corn (CK1) and the other group planting rice (PF1). We set up three sets of parallel experiments. CK0 were the original six soil samples, all from dryland. Three of them named CK0-D, were the soil samples before CK1 pot experiment as a control for CK1. The other three named CK0-P were the soil samples before PF1 pot experiment as a control for PF1. The potting device was a polyethylene (PE) bucket. The height of all buckets was 60 cm, and the thickness of the soil layer in the tank was 40 cm. When moving the soil, we did not disturb the soil and the original tillage layer was not damaged. The upper pot width of the corn pot was 45 cm, the width of the lower diameter was 40 cm. The upper pot width of the rice pot was 50 cm and the width of the lower diameter was 40 cm. The PF1 surface soil was maintained flat and moist, and the outdoor flooding was only conducted once the soil was stable and compact. On 13 June 2018, we planted corn into buckets, with five corn plants transplanted per bucket, row spacing 300 mm, and plant spacing 200 mm. On the same day, rice was transplanted into the bucket, with four rice plants transplanted per bucket, row spacing 300 mm, and plant spacing 200 mm. The rice was shallow-irrigated maintaining an average water layer of 40 mm, which is the recommended standard in North China. The irrigation water was tap water, in accordance with Chinese agricultural irrigation standards. One group was used for planting Nonghua 101 corn variety. The other group was submerged for five days and then planted with the rice Sterile Peduncle 31 variety (Figure 1).

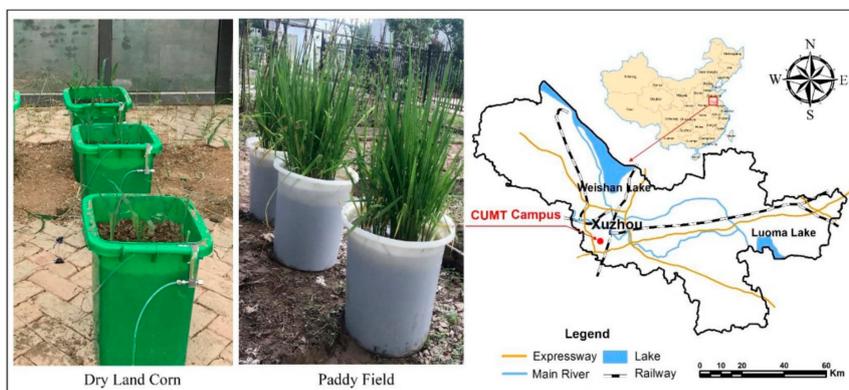


Figure 1. Location and photos of the experimental field.

2.2. Soil Sample Collection and Analysis

The first soil samples collected from two groups of experimental buckets on 13 June 2018, were labeled as CK0-D and CK0-P. Then, corn was planted in three experimental buckets (CK1), and rice was transplanted in another three experimental buckets (PF1). On 13 November 2018, a five-spot random sampling method was used to collect nearly 500 g of 0–20 cm mixed topsoil samples. In total, there were four groups with three repeats in each group for a total of 12 soil samples. The soil samples were sealed in sterile Ziploc (Hopebio Bio-Techonology Co., Ltd, Qingdao, China) bags and brought back to the laboratory immediately. One part of the soil samples was dried at room temperature for about 1 week, cleaned to remove gravel and animal and plant residues, then ground and filtered with

a 2-mm sieve to determine the basic physicochemical properties of the soil sample. Another part of the fresh soil samples was used directly for biodiversity analysis.

We choose soil pH, electrical conductivity (EC), SOM, ammonical nitrogen, nitrate nitrogen, available phosphorus (AP), and available potassium (AK) as factors to assess the impacts of the dryland-to-paddy conversion on soil ecological environment. These soil physicochemical properties are important indicators of soil fertility and soil nutrient cycling. At the same time, these indicators are also limiting factors for agricultural productivity under the land consolidation process in China [11,36]. Briefly, the soil pH and EC were measured using potentiometric methods (water:soil = 1:2.5) [34]. The SOM was determined by the potassium dichromate and external heating method [37]. The ammonical nitrogen was determined by potassium chloride extraction and distillation method [37], and the nitrate nitrogen was determined by the phenol disulphonic acid spectrophotometric method [37]. The AP was determined by the ammonium bicarbonate extraction and molybdenum antimony anti-colorimetric method [37], and the AK was determined by the ammonium acetate extraction and flame photometer method (FP640, Jingke, Shanghai, China) [37]. Each indicator per soil sample was measured three times, and the average value was calculated.

2.3. DNA Purification, PCR amplification, and High-Throughput Sequencing

Total DNA was extracted from the soil microorganisms using a FastDNATM SPIN Kit for Soil (MP Biomedicals, CA, USA) according to the kit instructions, generating a total of 12 DNA samples from the dryland and paddy fields. The concentration and purity of the total DNA extracts were determined using NanoDrop (ND-100, Molecular Devices, CA, USA). After the DNA extraction, the V4 and V5 regions of bacterial 16S rDNA were amplified using the primers 515F (5'-GTGCCAGCMGCCGCGTAA-3'), and 907R (5'-CCGTC AATTCMTTTRAGTTT-3'). The amplification of standard fungal ITS1 (internal transcribed spacer 1) was amplified using the primers ITS5F (5'-GGAAGTAAGTCGTAACAAGG-3'), and ITS1R (5'-GCTGCGTTCTTCTCGATGC-3'). The PCR amplification procedure was as follows: pre-denaturation at 98 °C for 2 min; denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, for a total of 25 cycles; a final extension at 72 °C for 5 min; and hold at 10 °C. The PCR amplification products were detected by gel electrophoresis on a 2% agarose gel, and the target fragments were recovered using a DNA gel extraction kit (Axygen, CA, USA). Based on the preliminary quantitative results of electrophoresis, the PCR amplification products were subjected to fluorescence quantification using a Quant-iT PicoGreen dsDNA Assay Kit (MP Biomedicals, CA, USA) and read using a quantitative instrumentation microplate reader (BioTek FLx800, Vermont, USA). Based on the fluorescence quantitation results, the samples were mixed in a ratio according to the sequencing amount required for each sample. A TruSeq Nano DNA LT Library Prep Kit (Illumina, CA, USA) was used to construct the soil microbial sequencing library. After quantification with Qubit and Q-PCR (quantitative real time polymerase chain reaction), the constructed library was sequenced with HiSeq2500 PE2500 (Illumina, CA, USA) [27].

2.4. Data Analysis and Processing

First, the raw sequence data were split and filtered to generate valid data. The qualified sequencing sequences are then diluted by the above-mentioned sequencing, mixed according to the required sequencing amount. Purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 300) on the Illumina MiSeq platform (Personalbio, Shanghai, China), according to the protocol. The sample sequencing data were distinguished with the barcode sequence, and the sequence of each sample underwent quality control. Then the non-specific amplification sequences and chimeric were removed with the usearch method (<http://www.drive5.com/usearch/>). Then, based on the valid data, the operational taxonomic unit (OTU) clustering and annotation were conducted to produce the OTUs, and the results of the basic analysis of the classification spectrum of each sample were obtained. A rarefaction curve for OTUs with 97% similarity was generated using the usearch method [17,34]. Bacterial 16S rRNA gene was identified using Greengenes database (Release 13.8,

<http://greengenes.secondgenome.com/>) [38]. The fungal ITS sequence of fungi uses the UNITE database (Release 5.0, <https://unite.ut.ee/>) [39].

The Simpson index, Chao 1 index, abundance-based coverage estimator (ACE), and Shannon index were used to demonstrate the richness and evenness of a single sample community. The α diversity was calculated through the galaxy platform (<http://mem.rcees.ac.cn:8080>). PCA was used to identify the primary characteristics of the soil microbial community distribution and to quantify the similarities and differences in microorganisms between groups, which is the β diversity. SPSS 20.0 (IBM, New York, USA) was used to conduct the Duncan analysis, which was used for multiple correlation analysis. Origin 9.0 (Origin Lab, Massachusetts, USA) was used to prepare the microbial community abundance plots at various levels. R-project (R Development Core Team, Vienna, Austria) was used to conduct the Mantel test. R vegan package (R Development Core Team, Vienna, Austria) was used to prepare the plots of microbial community structure and redundancy analysis (RDA) for the soil environment.

3. Results and Analysis

3.1. Impact of Dryland-to-Paddy Conversion on Soil Physicochemical Properties

We found no significant differences in the soil physiochemical properties between the wheat- and corn-planted soil samples (CK0-D, CK0-P, and CK1), indicating that their soil properties were similar. Significant differences were observed in some soil properties between the soil samples before and after the short-term land consolidation (CK0-P and PF1, respectively). The dryland-to-paddy conversion considerably impacted the soil properties. Except for EC, the soil pH, SOM, nitrate nitrogen, and ammonical nitrogen of PF1 were lower compared to those of CK0-P, and the standard deviations of these indicators for PF1 were less than those of CK0-P. When the indicators were considered individually, the dryland-to-paddy conversion impacted soil pH, EC, SOM, nitrate nitrogen, and ammonical nitrogen the most ($p < 0.05$). The soil acidity increased and the SOM decreased significantly after planting rice ($p < 0.05$). Conversely, the soil EC significantly increased after the conversion and was as high as $31.54 \text{ mS}\cdot\text{cm}^{-3}$ for PF1, significantly different from that of CK0-P by 45.31%. Soil available P and available K exhibited a decreasing trend that was not significant. In summary, continuous dryland planting did not significantly affect the soil properties. However, the standard deviation of soil property indicators was reduced after dryland-to-paddy conversion. We thought that short-term flooding increases the flow of soil nutrients, resulting in a homogeneous distribution. Thus, the conversion of dryland-to-paddy had some impact on the soil physicochemical properties in the short-term (Table 1).

Table 1. Description of soil physicochemical properties before and after the conversion of dryland to paddy.

Soil Properties	Dry Land Before Experiment		Dry Land Corn CK1	Paddy Field PF1
	CK0-D	CK0-P		
pH	$8.62 \pm 0.13\text{b}$	$8.43 \pm 0.23\text{b}$	$8.65 \pm 0.06\text{b}$	$8.15 \pm 0.01\text{a}$
EC ($\text{mS}\cdot\text{cm}^{-3}$)	$17.25 \pm 8.94\text{a}$	$16.85 \pm 4.96\text{a}$	$15.13 \pm 3.68\text{a}$	$31.54 \pm 2.72\text{b}$
SOM ($\text{g}\cdot\text{kg}^{-1}$)	$23.00 \pm 3.51\text{b}$	$21.55 \pm 7.12\text{b}$	$21.10 \pm 2.42\text{b}$	$13.05 \pm 1.19\text{a}$
NN ($\text{mg}\cdot\text{kg}^{-1}$)	$4.59 \pm 0.26\text{b}$	$4.14 \pm 0.43\text{b}$	$4.31 \pm 0.52\text{b}$	$3.30 \pm 0.27\text{a}$
AN ($\text{mg}\cdot\text{kg}^{-1}$)	$1.33 \pm 0.15\text{b}$	$1.33 \pm 0.17\text{b}$	$1.36 \pm 0.20\text{b}$	$0.93 \pm 0.07\text{a}$
AK ($\text{mg}\cdot\text{kg}^{-1}$)	$30.88 \pm 8.79\text{a}$	$30.41 \pm 8.67\text{a}$	$29.51 \pm 2.74\text{a}$	$23.25 \pm 1.58\text{a}$
AP ($\text{mg}\cdot\text{kg}^{-1}$)	$77.89 \pm 6.54\text{a}$	$76.22 \pm 4.79\text{a}$	$68.82 \pm 7.61\text{a}$	$76.59 \pm 2.62\text{a}$

Note: Data are mean \pm standard deviation. Values in the same row with the same letter(s) are not significantly different per the Duncan analysis at $p < 0.05$ between different treatments. SOM, soil organic matter; NN, nitrate nitrogen; AN, ammonical nitrogen; AP, available P; AK, available K. CK0-D is the soil sample before CK1 pot experiment as a control for CK1, CK0-P is the soil sample before PF1 pot experiment as a control for PF1, CK1 is the soil sample before corn harvest, and PF1 is the soil sample before rice harvest. The same below.

3.2. Impact of Dryland-to-Paddy Conversion on Soil Microbial Community

The study was conducted using the Illumina Miseq high-throughput sequencing platform. The OTUs at 97% similarity were statistically analyzed for microbial information. A total of 418,946 sequences of soil bacteria were detected from the 12 soil samples. There were 107,079, 101,975, 106,596, 104,194, and 103,296 valid sequences detected from CK0-D, CK0-P, CK1, and PF1, respectively. The bacterial average OTU numbers of the dryland CK0-D, CK0-P, and CK1 were 9138, 9241, and 9502, respectively, slightly higher than those of the paddy fields PF1 at 8086.

After processing and analysis, a total of 655,620 valid soil fungi sequences were detected. A total of 157,263, 138,888, 183,410, and 176,059 valid sequences were detected from CK0-D, CK0-P, CK1, and PF1, respectively. The fungal OTU numbers of the dryland CK0-D, CK0-P, and CK1 were 3236, 3787, and 3816, respectively, and those for the paddy field PF1 was 2191. The number of OTUs in all the PF1 samples at the phylum, class, order, genus, and species level were significantly lower than those in the drylands ($p < 0.05$). Preliminary analysis showed that after the dryland-to-paddy conversion, the soil fungal species decreased compared to the drylands. Thus, the conversion may inhibit the development of fungi in the short term.

3.2.1. Impact of Dryland-to-Paddy Conversion on Microbial Community Structure and Diversity

In this study, we used α diversities for analyzing the soil microbial richness and diversity. The Chao1 and ACE indices were used to reflect the community richness, and the Shannon index and Simpson index were used to reflect the community diversity. The α diversities of soil microorganisms before and after the land consolidation are listed in Table 2. In a short period, the microbial diversity significantly decreased after the dryland-to-paddy conversion. There was no significant difference in soil bacterial diversity between the dryland CK0-D, CK0-P, and CK1, although the microbial diversity indices of the paddy field PF1 reduced significantly. In detail, compared with CK0-P, the Simpson index decreased by 0.35–1.54%, and the Shannon index decreased by 3.62–13.18%.

Table 2. Impacts of the dryland-to-paddy conversion on soil microbial diversity.

Sequencing Type	Diversity Index	Dry Land Before Experiment		Dry Land Corn CK1	Paddy Field PF1
		CK0-D	CK0-P		
Bacteria	Chao1	2396 ± 348a	2528 ± 720a	2771 ± 654a	2297 ± 748a
	ACE	2472 ± 419a	2578 ± 725a	2818 ± 650a	2338 ± 757a
	Shannon	10.03 ± 0.20b	9.99 ± 0.30b	10.10 ± 0.24b	9.22 ± 0.43a
	Simpson	0.997 ± 0.00b	0.997 ± 0.00b	0.997 ± 0.00b	0.989 ± 0.07a
Fungus	Chao1	345.25 ± 116.2a	364.09 ± 104.8a	406.8 ± 83.0a	243.9 ± 66.8a
	ACE	345.45 ± 115.3a	364.05 ± 105.1a	407.1 ± 91.0a	244.5 ± 66.0a
	Shannon	6.28 ± 0.45b	6.12 ± 0.35b	5.64 ± 0.54b	4.56 ± 0.43a
	Simpson	0.955 ± 0.03b	0.957 ± 0.03b	0.933 ± 0.04ab	0.858 ± 0.68a

Note: Data are mean ± standard deviation. Values in the same row with the same letter(s) are not significantly different per the Duncan analysis at $p < 0.05$ between different treatments.

After the dryland-to-paddy conversion, the fungal Simpson index decreased by 5.97–14.62%, and the fungal Shannon index decreased by 21.38–32.07%, with an average decline of 25.49%. The conversion had a considerable impact on the Shannon index, and the difference was significant ($p < 0.05$). The soil microbial evenness decreased after the conversion as did the diversity and richness of soil bacteria and fungi in the short term. We found that the bacterial diversity was significantly changed compared to fungal diversity. Possibly because from the sequencing data, the unclassified sequence in the fungus was higher than the bacteria. And fungal classification and identification techniques are weaker than bacteria according to current sequencing technology.

PCA was conducted to assess the community composition of each sampling site at the genus level before and after the soil conversion. The results are shown in Figure 2. Points colored differently indicate

different samples (groups). The closer the two points, the more similar the microbial community structure, and smaller the difference. The PCA demonstrated that the microbial community in PF1 was different from that of the other three groups, whereas the soil bacterial composition structure was very similar between the two CKs.

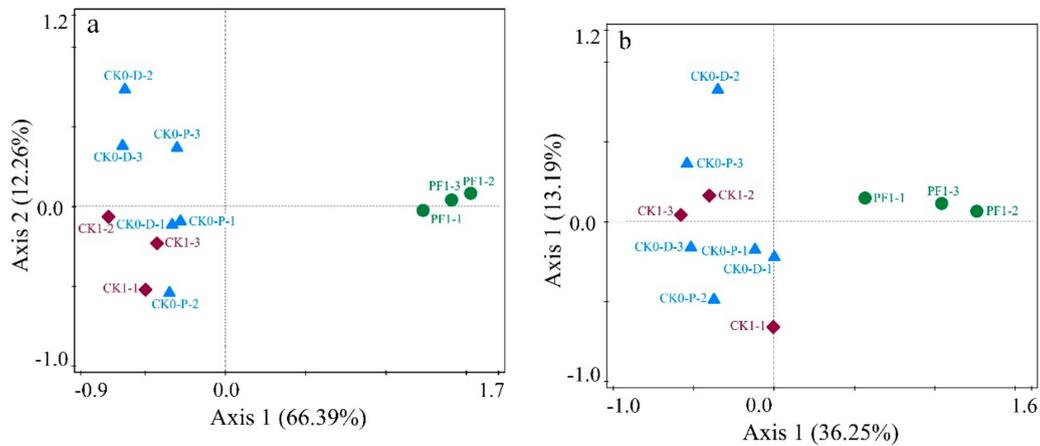


Figure 2. Principal component analysis (PCA) of soil (a) bacteria and (b) fungi at the genus level in the dryland-to-paddy conversion process. CK0-D is the soil sample before CK1 pot experiment, CK0-P is the soil sample before PF1 pot experiment, CK1 is the soil sample before corn harvest, and PF1 is the soil sample before rice harvest.

3.2.2. Impact of Dryland-to-Paddy Conversion on the Soil Microbial Community Composition

In the tested soils, 95% of the sequences from all the samples could be clearly classified. A total of 38 bacterial phyla were identified, of which the nine most abundant phyla accounted for more than 90% of the total relative abundance. These nine phyla included *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Planctomycetes*, and *Verrucomicrobia*, of which the *Proteobacteria* was the most abundant at 32.78% (Figure 3).

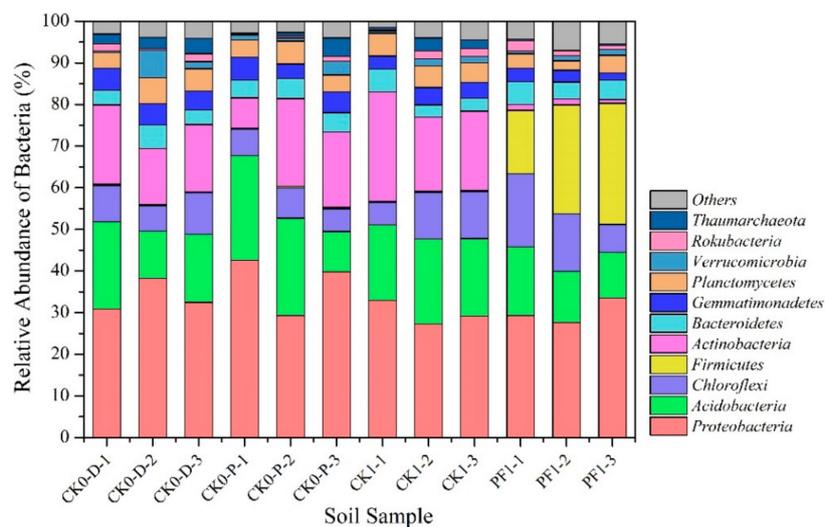


Figure 3. Changes in the relative abundance of bacterial phyla in the dryland-to-paddy conversion process.

Although we observed no remarkable difference in bacterial abundance between CK0-D, CK0-P, and CK1, the phylum composition of PF1 was different from the CKs, indicating that the land consolidation impacted the composition of the bacterial community. *Actinobacteria* favor neutral or

slightly alkaline and aerated soils [26,40]. Compared to the loose drylands, the soils of PF1 provided a significantly anaerobic environment after the conversion of dryland to paddy. The relative abundance of bacterial phyla changed considerably after the conversion of dryland to paddy. The *Actinobacteria* completely disappeared while the Firmicutes appeared in the PF treatment. The relative abundance of *Actinobacteria* declined from 16.27% before planting rice to 1.23%, a 13.23-fold decline. Under the submerged anaerobic environment after the conversion, the relative abundance of anaerobic phyla like *Chloroflexi* and *Firmicutes* in PF1 increased, with the most significant change in *Firmicutes*, increasing from 0.23% to 23.50% during the conversion.

At the phylum level, 14 and 16 fungal phyla were identified from the drylands and paddy fields, respectively. The relative abundance of the six major phyla, including *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Rozellomycota*, *Chytridiomycota*, and *Olpidiomycota*, accounted for 77.82%, among which the most abundant was *Ascomycota* at 33.01% (Figure 4). Similar to the bacterial abundance, the phylum composition of PF1 was significantly different from those of CK0-D, CK0-P, and CK1. Under the submerged environment after the conversion, the relative abundance of aerobic fungi like *Ascomycota* and *Chytridiomycota* decreased. Among them, we found a relatively exclusive appearance of the *Chytridiomycota* in CK0-D-1. The relative abundance of fungi was different from the other two CK0 treatments. The CK0-D-1 sample after planting corn was recorded as CK1-1, in which “Others” accounted for a large proportion. The relative abundances of *Ascomycota* in drylands and paddy fields were significantly different at 17.67% and 18.33%, respectively, after the conversion of dryland to paddy. Among them, the increase in *Olpidiomycota* was the most significant. With a relative abundance of 0.07% as a rare fungal phylum in CK0-D, the abundance of *Olpidiomycota* changed drastically to 10.00% in PF1 as a dominant phylum and can therefore be considered a sensitive phylum for studying the succession of microbial diversity. Previous studies have shown that soil pH and SOM were the dominant factors affecting the microbial community structure and diversity [41,42]. Hence, the differences in the microbial community structure of the 12 samples were probably related to the soil environmental factors.

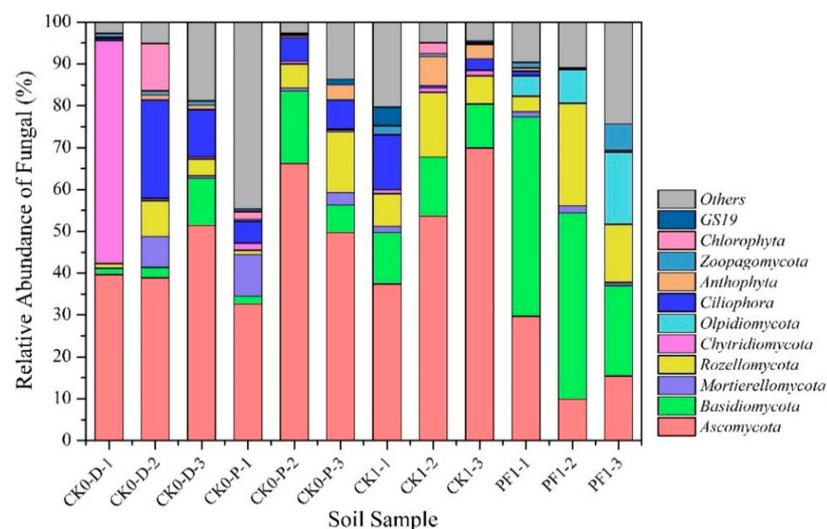


Figure 4. Changes in the relative abundance of fungal phyla in the dryland-to-paddy conversion process.

3.3. Impact of Soil Physicochemical Properties on the Microbial Community Structure of Consolidated Soil

To determine the chief environmental factors driving the microbial community, the Mantel test was conducted for the soil microorganisms and for the soil properties of the corresponding sampling sites before and after the conversion at the OTU level (Table 3). The structure demonstrated that the microbial population was correlated with EC and pH at high significance ($p < 0.05$). Microbial population was significantly correlated with pH, and bacteria and fungi the correlation coefficients

were 0.538, and 0.367 (Table 3). As an important indicator of soil salinity and acid-base level, soil pH had a significant impact on the differentiation of bacterial and fungal community structures. The other factors significantly affecting the microbial community structure for both bacteria and fungi were SOM, nitrate nitrogen, ammonical nitrogen, and the soil nutrient index.

Table 3. Mantel test analysis of microorganisms and soil environment factors at the operational taxonomic unit (out) level.

Bacteria			Fungi		
Variable	r	p	Variable	r	p
pH	0.538	0.004 **	pH	0.367	0.013 *
EC	0.576	0.002 **	EC	0.484	0.004 **
SOM	0.438	0.006 **	SOM	0.345	0.014 *
NN	0.359	0.021 *	NN	0.408	0.004 **
AN	0.351	0.012 *	AN	0.393	0.007 **
AP	−0.282	0.967	AP	−0.202	0.912
AK	0.253	0.039 *	AK	0.167	0.126

Note: ** and * mean significant difference at 5% and 10%, respectively. SOM, soil organic matter; NN, nitrate nitrogen; AN, ammonical nitrogen; AP, available P; AK, available K.

To further study the relationship between soil microbial phyla and soil properties, RDA was conducted, and the results are shown in Figures 5 and 6. Axis 1 and Axis 2 of the bacterial RDA were 68.43% and 14.09%, respectively, and Axis 1 and Axis 2 of the fungal RDA were 37.25% and 14.50%, respectively, which are both highly interpretable. The sampling points from the consolidated PF1 group behaved remarkably different from the other sampling points. All sampling points of PF1 demonstrated clear clustering with well-separated clusters. Both the bacterial and fungal communities in the drylands and paddy fields exhibited significant correlations with pH and EC. Except for EC, all relevant environmental factors were negatively correlated with the soil conversion of dryland to paddy.

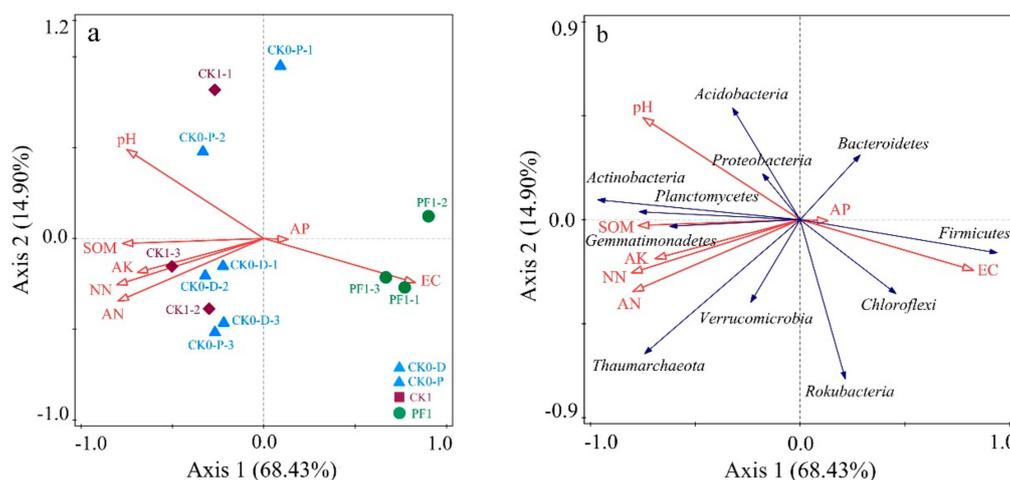


Figure 5. RDA (redundancy analysis) of (a) soil samples and the environmental factors and (b) chief bacterial phyla and the environmental factors.

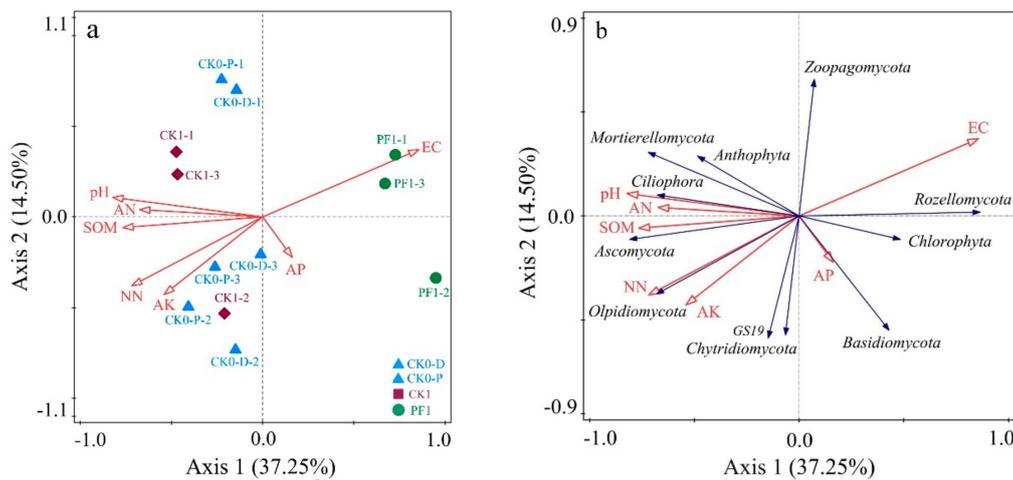


Figure 6. RDA of soil fungal community structure and soil environmental factors: (a) soil samples and the environmental factors; (b) chief fungal phyla and the environmental factors.

When the bacterial community of the soil (Figure 5a) was considered, EC lay on the second axis, preferably explaining the differences in community between drylands and paddy fields. Besides pH and EC, SOM, nitrate nitrogen, and ammonical nitrogen were also important factors affecting the microbial community structure. The correlation is indicated by the cosine value of the angle between a species and an environmental factor. As shown in Figure 5b, the soil EC had a highly positive correlation with *Firmicutes*, which was negative with *Actinobacteria* and *Planctomycetes*. The soil pH exhibited a strongly negative correlation with *Chloroflexi*. These results may explain the increase in EC and decrease in pH and SOM after land consolidation, resulting in dramatically decreased *Actinobacteria* and a substantial increase in *Chloroflexi* and *Firmicutes*, thus causing differences in the bacterial community.

Upon analyzing the soil fungal community, the pH, EC, SOM, nitrate nitrogen, and ammonical nitrogen of soil were found to be positively correlated with the relative abundance of the community structure. The soil EC is represented by the longest arrows in Figure 5, indicating that, as an essential environmental factor, EC led to community differentiation. The first axis explains the differences in the community structure of the drylands and the paddy fields. As shown in Figure 6, soil EC was correlated positively with *Chlorophyta* and *Rozellomycota*, and negatively with *Olpidiomycota*. Soil pH, SOM, and nitrate nitrogen were correlated positively with *Ascomycota*, *Ciliophora*, and *Mortierellomycota*. The differences in the soil physicochemical properties caused by soil environmental changes because of the conversion of dryland to paddy resulted in a decrease in the abundance of *Ascomycota* and *Mortierellomycota* and a substantial increase in *Rozellomycota*, driving the diversity differences in the fungal community.

4. Discussion

4.1. Impact of Conversion of Dryland to Paddy on Soil Eco-Environment

Land consolidation has now become China's largest undertaking to change the land use structure, affect farmland ecological services [36,43] and is essentially a process of ecological reconstruction. A slight change in the soil environment may lead to profound changes in the global environment [44]. The conversion of dry agricultural lands to paddy agriculture has become a common practice under the land consolidation policy. Changes in planting method and tillage practices would have a considerable impact on soil micro-ecology [45]. The soil hydrothermal and aeration conditions change with the conversion of dryland-to-paddy, thereby affecting the mineralization and humification of SOM. Water is the carrier of soil salt, and flooding treatment has a significant impact on EC. The leveling and merging of land and various field management measures are all considered important factors stimulating the

soil micro-ecological changes in the conversion of dryland to paddy [25,46]. Brye et al. reported a major change in soil physicochemical properties as a result of land consolidation, consistent with our results [13]. As mentioned in Table 1, the soil physicochemical properties before and after the conversion differed significantly. The pH range of CK0 were 8.42–8.74, extremely significantly different from the paddy fields after the conversion ($p < 0.001$). The rice and paddy fields produce more CO₂ and CH₄. In general, the acid gases, like CO₂ and CH₄, had the most obvious effect on soil pH in accordance with the conclusion of Oppermann et al., who stated that the introduction of acid gases would cause the pH to drop [47].

In ecosystems, the cycling of elements like carbon and nitrogen is a coupled and complex process affected by various interactive factors [48]. In land consolidation, humans interfere with ecological processes and cause changes in soil physicochemical factors, thereby affecting the carbonitride cycling in an agroecosystem. Compared with dry land, paddy fields are submerged for a long time. Most of the soil voids are occupied with water, have low soil oxygen and redox potential, and reduced soil activity [18,49]. Therefore, we suggest that the reason that carbon, nitrogen, and phosphorus contents of paddy fields in this study were lower than those of dry land was the changes in the soil environmental conditions. Different tillage methods and management practices lead to changes in soil moisture and profound changes in the soil redox potential, affecting the form and availability of phosphorus in soil. In this study, the average AP contents in CK0-P and PF1 were 77.89 and 76.59, respectively, not consistent with the finding of Mulqueen et al. who stated that submergence was conducive to the release of soil phosphorus [50]. The primary reason may be the redistribution of soil phosphorus in plants-soil-microorganisms under different land-use patterns so that the soil AP was reduced after the dryland-to-paddy conversion.

4.2. Changes in Microbial Community Structure and Function After the Dryland-to-Paddy Conversion

Soil microorganisms are sensitive to changes in land use and the environment [51,52]. We found no significant changes in the microbial diversity of soils with dryland planting of corn. However, the microbial diversity significantly decreased in the short term after the conversion of dryland to paddy, and the Simpson and Shannon indices were all lower in PF1 than in three CKs ($p < 0.05$). Microorganisms in the soil have certain selective adaptability to the soil environment and are closely associated with its environmental factors [41,53]. The Mantel test and RDA results showed that the change in planting method altered the soil physiochemical properties after the conversion, which led to significant changes in the structure of the soil microbial community. Among the soil physiochemical factors, soil pH and EC were dominant, affecting the microbial community. Changes in soil moisture altered the oxygen condition of the soil. Therefore, the submerged rice fields had lower oxygen concentrations, inhibited growth of soil aerobes, and enhanced survival of anaerobes and facultative anaerobes, such as denitrificans and methanogens, leading to variations in microbial community composition and diversity [54]. Likewise, Drenovsky et al. found huge differences in the soil microbial community structure between aerobic and submerged conditions, consistent with our results [42]. Sahrawat et al. found that submerged conditions were not conducive to the mineralization and transformation of soil organic carbon and suppressed the carbon supply capacity of the soil, thus affecting the soil microbial composition [55].

Bacteria are the most diverse microbial populations in soil and are extremely sensitive to environmental variations [56]. Similar to most agricultural soils [15], the phylum *Proteobacteria* was the most dominant at 32.78%. The primary function of *Proteobacteria* is organic nitrogen fixation, and can adapt well to various complex environments [57]. *Actinobacteria* grow vigorously in environments with higher OH⁻ concentrations [40]. After the conversion, the pH of the paddy field was lower than that of the dry land; therefore, *Actinobacteria* richness was significantly higher in the consolidated paddy fields. *Chloroflexi* and *Firmicutes* are often detected in anaerobic sludge [58]. After the conversion of dryland to- paddy, *Chloroflexi* showed a significantly increased relative abundance. *Chloroflexi* are facultative anaerobes that can produce energy through photosynthesis using CO₂ as a carbon source, generating

no oxygen in the process, and converting CO₂ into soil inert carbon at lower nutrient conditions [58]. The dramatic changes in the farmland soil environment before and after the land consolidation greatly affected the soil carbonitride cycling of which microorganisms are a part. *Firmicutes* are anaerobic redox bacteria. Their growth is positively related with temperature within a certain range [59]. Under the submerged conditions after the conversion of dryland to paddy, the small differences between day and night temperatures, with higher average temperature than that of the drylands in the same period, may be the cause of the significantly higher abundance of *Firmicutes* in the paddy fields than in the drylands [59].

The composition of soil fungi dominant bacteria had considerably changed after the dryland-to-paddy conversion. In the CK0-D, CK0-P, and CK1 groups, *Ascomycota* was the most dominant phylum at 48.82% significantly different from PF1. The most dominant phylum in PF1 was *Basidiomycota*, which mostly grow in damp environments of rotten woods and compost piles [60]. This also implied that the change of soil anaerobic environment after the conversion of dryland to paddy was more conducive to the growth of *Basidiomycota*. We found that soil nitrogen content in PF1 was less than the three CKs, and this was different from the change of *Basidiomycota*. However, Sterkenburg et al. [61] found that *Basidiomycota* are positively correlated with the soil nitrogen content, inconsistent with our findings. We supported that *Basidiomycetes* are affected by many factors, such as soil temperature, humidity, and physicochemical properties [15,60]. Not only because of changes in soil conditions, but also the role of microbes is subtle [27,56]. In this study, the composition of the soil microbial community and diversity index of fungi are different from those in dry land. This may be due to differences in the microbial community structure and a combined result of various soil environmental factors [41,60]. Fungi constitute most of the soil microbial biomass, are the bearers of material circulation and energy exchange playing an important role in the carbonitride cycle [16]. Because of the differences in fungal composition, organic matter decomposition in dry land and paddy fields may have a large difference. In this study, 13.07% of sequences were unclassified, indicating that many fungal species need to be further identified and classified.

4.3. Uncertainty of Ecological Impacts of Dryland-to-Paddy Conversion

To comply with the farmland construction standards and with the policies of “compensating excellent land for excellent land taken” and “compensating paddy fields for paddy fields taken,” the conversion of dryland to paddy has become common in China’s land consolidation. By adjusting the existing cropping patterns and a series of engineering measures for field leveling, irrigation facilities, and soil, the original low-yielding, low income, and disaster-prone drylands can be transformed into high-quality better-yielding paddy fields. As an important source of greenhouse gas emissions, rice fields seriously threaten global climate changes [62]. Whereas the conversion of dryland to paddy ensures food security, further global climate change challenges could be created. The global warming potential (GWP) of CH₄ is 25-fold of that of CO₂, and the GWP of N₂O is 298-fold of that of CO₂ [63]. Some scholars have shown that because paddy fields are favored, the growth of anaerobic methanogens promote the emission of CH₄ [64–66]. In addition, compared with dry land, rice fields produce more N₂O [67]. Large areas of conversion of drylands to paddy fields may ensure the safeguard food security, but may lead to the production of greenhouse gases and potentially create ecological risks.

Since sunlight, temperature, and water requirements of various crops are different, paddy rice can only be cultivated in areas with good hydrothermal conditions. In response to the requirements of national macroeconomic policies, the North China Plain and the Northeast Plain have extensively undertaken dryland-to-paddy conversion projects. As a result, well-irrigated rice fields are common, severely challenging the safety of groundwater resources [68]. The groundwater consumption by agricultural practices in the North China Plain is the primary reason for the decline in the groundwater level and the formation of groundwater funnels in the past 40 years [22,69]. The paddy fields in the Northeast Plain have increased by 120% (from 2.5 to 5.5 million hectares). The continuous expansion

of paddy fields is posing enormous ecological risks to the northeast region, which has limited water resources and a relatively fragile wetland ecosystem [70].

The large-scale expansion of agricultural irrigation has led to changes in surface radiation and energy balance, thus affecting the ground humidity and temperature [71–73]. Kang and Eltahir [28] showed that the expansion of irrigated agriculture in the North China Plain has facilitated surface evaporation, increased the exposure of the surface to radiation, and affected the surface temperature and humidity, thereby increasing the intensity of heat waves, intensifying summer high-temperature extremes, and accelerating global climate change. The large-scale expansion of dryland-to-paddy conversion has increased pressure on regional water resources, increased environmental changes, and the resulting ecological problems need to be fully evaluated. In the future, water-efficient irrigation should be aggressively used to improve the efficiency of water-use, thus enabling the sustainable development of land and water resources. Conversely, effective emission reduction measures based on various factors should be undertaken, and temporospatial dynamic monitoring should be used to evaluate the ecological effects because of the dryland-to-paddy conversion. These will help encourage low-carbon agriculture and promote food security and the sustainable development of arable land, which are essential for mitigating the challenges of global climate change.

5. Conclusions

Land consolidation of dryland-to-paddy conversion for improving grain production capacity is widely implemented throughout the world. However, few studies have focused on the impact of this process on the soil environment. Therefore, a simulation experiment was used to determine the impacts of the dryland-to-paddy consolidation on soil microbial community. The results showed that soil pH, SOM, nitrate nitrogen, and ammonical nitrogen decreased and soil EC increased in a short time because of the conversion of dryland to paddy. After the dryland-to-paddy conversion, the soil microbial richness and diversity decreased, and the soil microbial community composition and relative abundance changed significantly in the short term. The relative abundance of *Actinobacteria*, *Firmicutes*, and *Olpidiomycota* varied widely and could be used as sensitive microorganisms indicating microbial diversity succession in the conversion. Moreover, the changes of soil environmental factors including pH, EC, SOM, nitrate nitrogen, and ammonical nitrogen were the primary reasons leading to the changes in the soil microbial community structure. Land consolidation of dryland-to-paddy conversion may alter the soil organic carbon decomposition and CH₄ emission. These potential negative impacts on soil environment should be paid attention to achieve sustainable intensive agriculture and mitigate aggravating global warming.

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