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Towards Improved Grain Yield and Soil Microbial Communities of Super Hybrid Rice through Sustainable Management

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Abstract: Superior yields of super hybrid rice have demonstrably contributed to contemporary food security. Despite this, the extent to which intensive nitrogen fertilizer requirements of such crops have impacted on soil health and microbial communities primarily remains unchartered territory, evoking questions of sustainability. Here, we examine how four management treatments (zero fertilizer, CK; farm practice, FP; high-yield and high-efficiency, HYHE; and super-high-yield management, SHY) influenced the grain yields, soil biodiversity and community strata underpinning soil health of an elite super hybrid rice variety (Y-liangyou 900). We show that SHY treatments increased yields, altered soil physicochemical properties, and fostered greater biodiversity and soil bacteria and fungi abundance, while FP, HYHE and SHY treatments transformed community bacteria and fungi strata. Environmental regulators of bacterial and fungal communities differed widely, with bacterial communities most closely associated with soil organic carbon (SOC) and NH_4^+-N, and with fungal communities more related to available phosphorus. We show that alpha diversity of bacteria and fungi and community composition of fungi were positively correlated with yield, but bacterial community composition was negatively correlated with yield. Our work clearly exemplifies the nexus between appropriate farm and landscape management in enabling soil health and driving consistently high yields, of which both are required for sustainable food security.

Keywords: super hybrid rice; management practices; soil microbial diversity; soil microbial community; high-throughput sequencing

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1. Introduction

As the world's largest producer of rice (*Oryza sativa* L.), Chinese rice production comprises a key pillar in global food security [1]. In pursuit of solutions that enable greater productivity without environmental cost, the Ministry of Agriculture in China launched the super rice breeding program in 1996. After approximately 30 years of research, the program successfully developed elite 125 super hybrid cultivars, wherein rice yields soared to unprecedented levels of 24 t hm^{-2} in 2021 [2,3]. Research and development of super hybrid rice has made an important contribution to China's food production, but extensive biomass of super hybrid rice necessitates substantial fertilizer input, which, if mismanaged, induces resource wastage, including nitrate leaching and nitrous oxide emissions, resulting in significant environmental costs [4,5]. It is thus imperative that sustainable management measures are developed, trialed and adopted.

Soil microbial communities represent one of the most diverse and abundant communities on Earth, playing vital roles with significant ecological, economic and societal implications. Many factors—including soil water management, nutrient management, and land use—are influenced by soil physical, chemical and biological properties [6,7]. The composition of soil, both chemically and microbiologically, is impacted by practices related to soil water management, nutrient management, and tillage patterns [8]. The physical and chemical characteristics of soil are closely intertwined with the activity of soil microorganisms. These microorganisms serve as critical indicators of changes in soil quality resulting from alterations in its physical and chemical properties [9].

Fertilizers are considered to be the main drivers of soil microbial community alteration [10]. The application of artificial fertilizers can enhance soil nutrient levels and modify the diversity of dominant microbial species. Consequently, this indirectly influences the physicochemical properties of the soil and leads to significant alterations in the composition and biomass of the microbial community [11]. Long-term modifications in the abundance and makeup of soil microorganisms have far-reaching implications for the physicochemical composition of the soil and the transformation of energy, nitrogen (N), phosphorus (P), and potassium (K) [12]. The consequences of unbalanced fertilization practices on the microbial community are manifold. For instance, phosphorus shortages promote the growth of Acidobacteriota and Chloroflexi, while nitrogen deficiencies increase the relative abundance of oligotrophic groups [13]. In contrast, the long-term and appropriate application of fertilizers at suitable ratios maintains soil pH stability, enhances soil microbial load, promotes microbial diversity, and preserves soil health. This approach proves crucial for sustaining the long-term fertility and overall well-being of the soil.

Moisture conditions play a vital role in shaping soil microbial activity and overall ecosystem functioning. These conditions not only affect the physical diffusion and chemical transformation of soil nutrients but also have a profound impact on the structure of soil microbial communities and the microbial-mediated carbon (C) and nitrogen cycles. Changes in soil moisture conditions can lead to significant alterations in the composition of soil fungal populations, thereby affecting the overall structure of the microbial community [14,15]. Water stress, for instance, has notable effects on both root microorganisms and soil enzyme activities, with mild water stress being particularly effective in promoting these responses [16]. Drought conditions in soil can bring about changes in microbial communities, whereas intermittent irrigation proves beneficial in enhancing the functional diversity of soil microorganisms in rice cultivation [17].

The problem of pursuing a high yield with large amount of fertilizer and water in the production of super hybrid rice exists, and the effect of using reasonable fertilizer application on soil physicochemical properties and microorganisms of super rice has been studied, but less research has been conducted on different management practices. The use of reasonable cultivation practices can promote rice yield increase, improve soil health, and enhance soil microbial diversity. In this study, a locational field experiment was conducted from 2017 to 2022 to investigate the effect of different management practices on soil physicochemical properties and soil microbial community diversity and structure of super hybrid rice. It is of significant practical significance to improve the high-yielding and efficient production of super hybrid rice, which also further ensures food security and the sustainable development of agriculture.

2. Methods and Materials

2.1. Experimental Site Description

The field experiment was conducted in Jingzhou, Hubei Province, China from 2017 to 2022 (112°15′ E, 30°30′ N). The local climate is subtropical monsoonal humid climate. Annual sunshine hours were 1800–2000 h. The average annual temperature was 15.9−16.6 °C, the annual accumulated temperature ≥ 10 °C was 5000–5350 °C, the annual frost-free period was 242–263 days, and the annual rainfall was 1200–1700 mm. The experimental soil was a purple tide mud rice soil developed from Yangtze River sediments. Before the

experiment, the soil of the experimental site had the following properties: pH 7.0; total nitrogen (TN) 3.12 g kg^{-1}; available P (AP) 22.89 mg kg^{-1}; available K (AK) 92.2 mg kg^{-1}; organic carbon (SOC) 14.52 g kg^{-1}.

2.2. Experimental Design

The rice variety used in the present study was Y-liangyou 900 (YLY900). YLY900 is an indica hybrid variety with Y58S as the female parent and R900 as the male parent, using the two-line method. Treatments were set up at zero-fertilizer control (CK); farmers' practice (FP); high-yield and high-efficiency management (HYHE); and super-high-yield management (SHY). Management methods were based on a combination of nitrogen fertilization, planting density and water management practices. No fertilizer was used in the CK treatment. The FP treatment received a total N rate of 210 kg ha^{-1}, of which 70% was applied as basal dressing and 30% at tillering. Phosphorus was applied as basal fertilizing with 90 kg P ha^{-1} as urea and potassium with 112.5 kg K ha^{-1} as potassium chloride, equally divided between the basal and the emergence stage in the FP treatment. In the HYHE treatment, a total N rate of 195 kg N ha^{-1} was used and N was applied at the basal, mid-tilling and panicle emergence stages in a ratio of 5:2:3. The way phosphorus and potassium fertilizers were managed in the HYHE treatment was the same as in FP. An additional 5 kg $ZnSO_4$ ha^{-1} zinc fertilizer was applied in the HYHE treatment. The total N application rate was 270 kg N ha^{-1} in the SHY treatment. N applications were 50%, 20%, 20% and 10% at basal, mid tillering, panicle initiation and top dressing. In the SHY, FP, and HYHE treatments, phosphorus fertilizer management was consistent. In the SHY treatment, potassium chloride was applied at 150 kg K ha^{-1}, equally divided between the basal and panicle initiating stages. For HYHE and SHY, the planting density was 30 cm by 16 cm, while for CK and FP, it was 30 cm by 18 cm. A seed bed was used to plant the pre-germinated seeds [18]. The transplanting of the seedlings to the field plots, with two seedlings per hill, took place between 28 and 32 days after the planting. The transplantation dates were 3 June 2017, 1 June 2018, 10 June 2019, 10 June 2020, 3 June 2021, and 3 June 2022 in Jingzhou.

With regard to water management, all treatments were submerged for 5 days after transplanting and continued to be submerged in shallow water until 5 days prior to panicle emergence. The HYHE and SHY studies utilized the technique of shallow water deep wetting and drying (SWD). SWD involved watering the plots to a depth of 3.0 cm, allowing to dry and then watering again to the same depth before visible cracking had occurred on the soil surface. Fields were shallow wetted and dried until one week before harvest and then dehydrated for HYHE and SHY. Throughout the growing cycle, insects and diseases were chemically controlled [3]. Cultivation management practices were consistent between 2017 and 2022. Detailed cultivation practices are shown in Table 1.

Table 1. Water, spacing, fertilizer management of experimental field from 2017 to 2022.

Treatment	Fertilizer	Spacing	Water Management
	N-P-K-Zn-Si	(cm)	
CK	0-0-0-0-0	30 × 18	Continuous flooding; field drained after flowering
FP	210-100-220-0-0	30 × 18	Continuous flooding; field drained after flowering
HYHE	195-100-180-5-0	30 × 16	SWD; Dehydrate 1 week before harvest
SHY	270-120-240-5-150	30 × 16	SWD; Dehydrate 1 week before harvest

2.3. Sample Collection and Analyses

2.3.1. Soil Sample Collection

After rice harvest in 1 October 2022, soil samples at the depth of 20 cm were randomly collected at 5 points in each treatment with a soil drill with 0–20 cm diameter. The soil samples of five soil samples were mixed, and after removing stone, root, and plant residues with 2 mm mesh, each sample was divided into two parts: half plastic was used for

physical and chemical analysis of fresh soil, and half air-dried was used for physical and chemical analysis. The treated soil samples were divided into three replicates for experimental analysis.

Before collecting the samples of soil, the sampling instruments were sterilized beforehand, the top layer of floating soil was removed during sampling, and a 5–20 cm layer of soil was dug with an ethanol-fired shovel. After removing visible impurities, the soil was passed through a 2 mm sieve, and each sample was collected and mixed from 5 sampling points. The samples were stored in 5 mL centrifuge tubes after removing impurities, transported back to the laboratory at 0 °C, and then stored at −80 °C for DNA extraction.

2.3.2. Determination of Soil Physicochemical Indexes

The air-dried soil samples were used to determine physicochemical properties. Soil pH was measured in water (1:2.5 w/v) by a pH meter. Soil total N and total C were determined via element analyzer. Determination of NH_4^+-N by 2 mol · L KCL extraction-indophenol blue colorimetric method. NO_3^--N was determined via dual-wavelength ultraviolet spectrophotometry. TP and TK were determined via sodium hydroxide (NaOH) melting-molybdenum-antimony resistance colorimetric method and flame photometry. Determination of AP was carried out via molybdenum-antimony resistance colorimetry method. AK was measured via a photoelectric flame photometer. The SOC was determined using a wet oxidation procedure with potassium dichromate ($K_2Cr_2O_7$)-sulfuric acid (H_2SO_4).

2.3.3. Grain Yield

During the rice maturity period from 2017 to 2022, grain yield was determined from a 5 m^2 area in each plot and standardized to a moisture content of 0.14 g H_2O g^{-1}. At the same time, the 12 diagonal hills of the square measuring area were taken for seed testing to examine traits such as effective panicles, spikelets per panicle, and grain weight.

2.3.4. Soil DNA Extraction and High-Throughput Sequencing Analysis

The V34 hypervariable region of bacterial 16S rRNA gene sequence and the ITS1-5F region of fungal rRNA gene sequence were amplified via Illumina NovaSeq sequencing platform. The promoter library was constructed by using labeled universal primers for bacteria and fungi, that is, 341F and 806R of bacteria and ITS5-1737F and ITS2-2043R of fungi. The DNA samples were amplified individually using the primer pairs, 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT), for bacteria and ITS5-1737F (GGAAGTAAAAGTCGTAACAAGG) and ITS2-2043R (GCTGCGTTCTTCATCGATGC) for fungi to generate polymerase chain reaction (PCR) fragments. The following hot procedures were used for amplification: initial denaturation of 98 °C 2 min, then denaturation at 98 °C for 15 s, then denaturation at 55 °C for 30 s, extension at 72 °C for 30 s, and finally, extension of 5 min at 72 °C. The test was carried out by Beijing Nuohe Zhiyuan Company on the Illumina NovaSeq sequencing platform.

2.4. Sequence Processing

The original data of each sample were obtained according to barcode, and the barcode and primers were removed, and then the R1 and R2 sequence data were spliced by FLASH software. The spliced Tags were quality controlled to obtain Clean Tags, and then chimera filtering was performed to obtain valid data (Effective Tags) that could be used for subsequent analysis. Small fragment libraries were constructed based on the characteristics of the regions amplified, and the libraries were double-end sequenced based on the Illumina NovaSeq sequencing platform. For the double-ended reads obtained via sequencing, splicing, filtering, and noise were performed using DADA2 pipeline, followed by species annotation (ASV analysis) as well as abundance analysis of the validated data obtained, leading to further alpha diversity and beta diversity analysis.

Excel 2019 and Statistix 9 software were used to analyze the variance of yield, soil physical and chemical properties and microbial diversity. Based on Bray–Curtis distance measurement and abundance data, the structural differences of bacterial and fungal communities under different treatments were determined, and the linear discriminant analysis effect size (Lefse) algorithm was used to determine which taxa were significantly affected. The physical and chemical properties of soil and the main taxa of main bacteria and fungi were analyzed. The partial least squares path model (PL–PM) was used to evaluate the effects of soil characteristics, α diversity and microbial community on rice yield by using goodness-of-fit (GOF) statistics.

3. Results

3.1. Soil Properties

The results showed that there was no significant difference in soil pH among different soil treatments (Table 2), but there were significant differences in NH_4^+-N, NO_3^--N, TK, AK, TP, AP and SOC among different treatments ($p < 0.05$). The contents of TN and AK in SHY treatment were the lowest and significantly lower than those in the other three treatments, while the contents of NH_4^+-N, TP, AP, and C/N were the highest and significantly higher than those in the other three treatments. The NO_3^--N of the HYHE treatment was significantly higher than that of the other three treatments, and the order was HYHE > FP > CK > SHY. The content of SOC under the HYHE treatment was significantly higher than that of the other three treatments, and there was no significant difference among the other three treatments.

Table 2. Basic physical and chemical properties of soil under different management practices.

Soil Properties	Treatment			
	CK	**FP**	**HYHE**	**SHY**
pH	7.25 ± 0.15 a	7.37 ± 0.05 a	7.39 ± 0.09 a	7.35 ± 0.16 a
TN ($g \cdot kg^{-1}$)	3.75 ± 0.06 a	3.69 ± 0.22 a	3.62 ± 0.06 a	3.19 ± 0.09 b
NH_4^+-N ($mg \cdot kg^{-1}$)	3.08 ± 0.12 c	3.05 ± 0.04 c	3.31 ± 0.09 b	4.38 ± 0.16 a
NO_3^--N ($mg \cdot kg^{-1}$)	3.95 ± 0.12 b	3.96 ± 0.14 b	4.48 ± 0.14 a	3.15 ± 0.10 c
TK ($g \cdot kg^{-1}$)	185.36 ± 5.67 b	204.9 ± 1.40 a	207.47 ± 4.25 a	212.47 ± 4.81 a
AK ($mg \cdot kg^{-1}$)	82.19 ± 0.65 b	81.33 ± 1.80 b	87.44 ± 1.29 a	78.62 ± 0.28 c
TP ($g \cdot kg^{-1}$)	3.28 ± 0.13 c	5.81 ± 0.14 b	5.73 ± 0.22 b	7.4 ± 0.030 a
AP ($mg \cdot kg^{-1}$)	20.76 ± 0.10 c	23.69 ± 0.11 a	23.16 ± 0.14 b	23.85 ± 0.16 a
SOC ($g \cdot kg^{-1}$)	15.73 ± 0.19 b	15.21 ± 0.52 b	16.52 ± 0.17 a	15.59 ± 0.40 b
C/N	4.19 ± 0.02 c	4.16 ± 0.19 c	4.56 ± 0.11 b	4.88 ± 0.04 a

Note: TN, total nitrogen; NH_4^+-N, ammonia nitrogen; NO_3^--N, nitrate nitrogen; TK, total potassium; AK, quick-acting potassium; TP, total phosphorus; AP, available phosphorus; SOC, organic carbon. Within a row, data (mean ± SD, n = 3) followed by different letters are significantly different ($p < 0.05$).

3.2. Alpha Diversity of Bacterial and Fungal Communities

After quality control, each sample contained 62,288–76,393 sequences and 464–2188 read numbers, and the sample sequencing coverage was 100%. Among the four treatments, the SHY treatment had the highest number of ASVs for both bacteria and fungi. In the FP treatment, fungi had the lowest number of ASVs.

The richness and diversity index shown in Table 3 showed that except for Simpson index, there were significant differences in bacterial and fungal alpha diversity among different treatments. The bacterial group exhibited significantly higher Ace index and Chao1 index in SHY treatment and HYHE treatment than in CK and FP treatment, while Shannon index was not significantly different from CK and FP, indicating that HYHE treatment had a significant effect on the abundance of soil bacteria, but not on diversity. The richness and diversity of fungal treated with FP were significantly lower than those of other treatments, indicating that FP treatment significantly reduced the richness and diversity of fungal in rice fields. Among the treatments, the Ace index and Chao1 index of

SHY treatment were the highest, indicating that the population richness of bacterial and fungal in paddy soil was the highest in long-term, super-high-yield treatment.

Table 3. The alpha diversity of bacterial and fungal gene sequences under different management practices measured by Ace (a), Chao1 (b), Shannon (c), and Simpson (d) indices, respectively.

Microbe Type	Treatment	Richness Index		Diversity Index	
		ACE	Chao1	Shannon	Simposn
Bacteria	CK	1827 ± 46 b	1827 ± 45 b	10.19 ± 0.08 b	0.999 ± 0.001 a
	FP	1934 ± 9 ab	1977 ± 93 ab	10.29 ± 0.08 ab	0.999 ± 0 a
	HYHE	2064 ± 24 ab	2034 ± 51 a	10.28 ± 0.10 ab	0.999 ± 0.001 a
	SHY	2188 ± 81 a	2121 ± 115 a	10.41 ± 0.09 a	0.999 ± 0 a
Fungi	CK	495 ± 4 b	495 ± 15 bc	6.16 ± 0.34 a	0.958 ± 0.015 a
	FP	464 ± 6 c	464 ± 20 c	5.68 ± 0.18 b	0.917 ± 0.049 a
	HYHE	511 ± 8 b	511 ± 27 b	6.34 ± 0.10 a	0.969 ± 0.007 a
	SHY	556 ± 10 a	556 ± 27 a	6.52 ± 0.17 a	0.969 ± 0.021 a

Note: Different letters for the same item indicate $p < 0.05$ (significant differences).

3.3. Beta Diversity of Bacterial and Fungal Communities

As shown by the Veen diagram (Figure 1a), the number of bacterial ASVs specific to the CK, FP, HYHE and SHY treatments were 898, 1153, 1190 and 1300, respectively, and the bacterial ASVs of the FP, HYHE and SHY treatments accounted for 23.1%, 24.9% and 24.1% of the CK treatment, respectively. From the Venn diagram (Figure 1b), the number of fungal ASVs specific to the CK, FP, HYHE and SHY treatments were 271, 230, 225 and 325, respectively, and the fungal ASVs of the FP, HYHE and SHY treatments accounted for 22.9%, 20.9% and 23.2% from the CK treatment, respectively. It shows that fungal and bacterial communities have both the same and unique components.

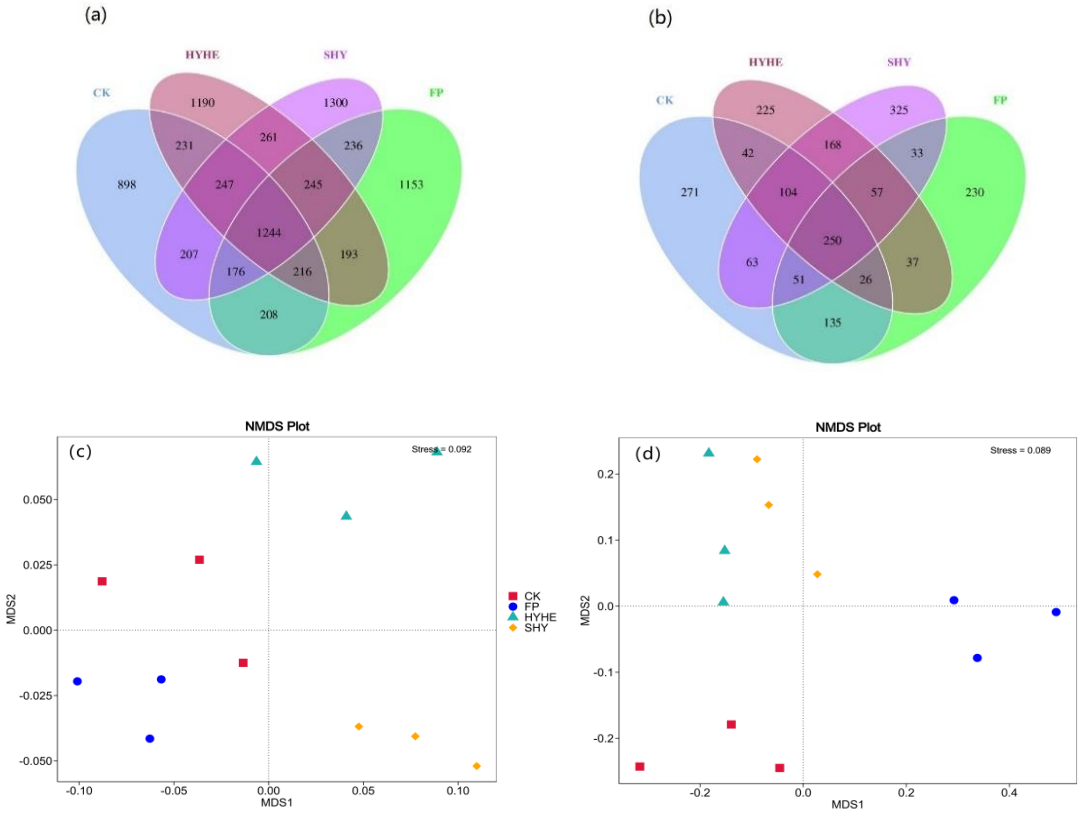

Figure 1. VEEN diagrams of working taxa of bacteria (**a**) and fungi (**b**) and NMDS analysis maps of bacterial (**c**) and (**d**) fungal based on Bray–Curtis differences in different management practices.

NMDS analysis showed that the stress of bacterial community was 0.092 and the stress of fungal community was 0.089, the stress of different microbial communities was less than 0.1, which indicated that the analysis results were reasonable and statistically significant. CK, FP, HYHE and SHY treatments were basically located in different quadrants, and the structural composition of the bacterial community varied widely (Figure 1c). HYHE and SHY treatments were located in the same quadrant, the similarity of fungal community structure is high, CK and FP are in different quadrants, and there are great differences between their groups (Figure 1d).

3.4. Composition of Bacterial and Fungal Communities

Proteobacteria was the bacterial community with the highest relative abundance at the gate level, as high as 22.5–24.6%, and the relative abundance of Actinobacteriota (8.6–13.3%), Acidobacteriota (10.6–13.2%) and Chloroflexi (8.2–10.2%) is relatively high. This was followed by Firmicutes (2.9–4.5%), Myxococcota (6.2–6.6%), Gemmatimonadota (3.9–5.4%), Desulfobacterota (3.6–4.8%), MBNT15 (3.8–4.7%) and Bacteroidota (2.5–3.6%), respectively (Figure 2a). The relative abundance of Proteobacteria and Gemmatimonadota treated with SHY was significantly higher than that of other treatments, while the relative abundance of Actinobacteriota, Chloroflexi and Firmicutes treated with SHY was significantly lower than that of other treatments. The relative abundance of Firmicutes bacteria treated in the HYHE was significantly higher than other treatments, while the relative abundance of Acidobacteriota bacteria was slightly lower than that of other treatments.

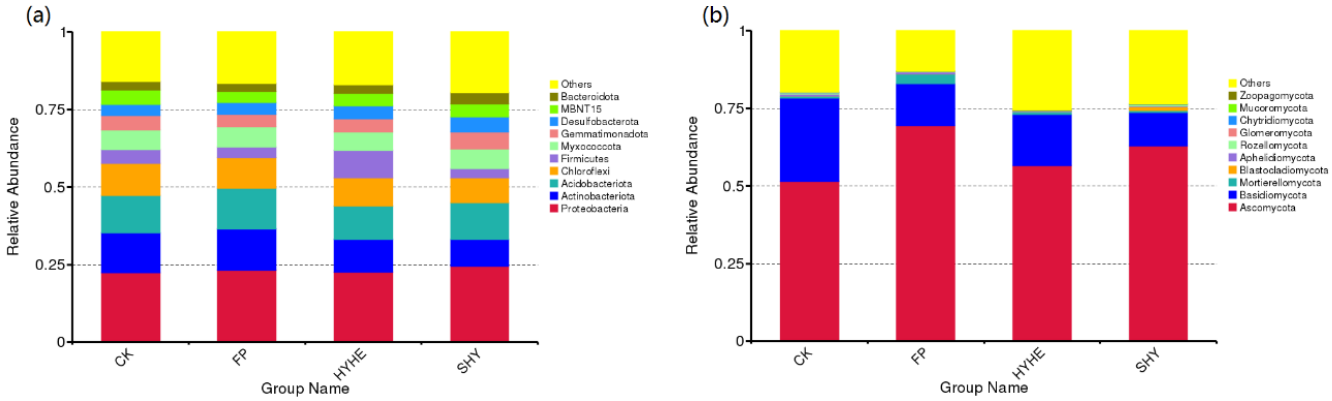

Figure 2. Relative abundance of main taxa of bacteria (**a**) and fungi (**b**) at phylum level under different management practices.

Ascomycota was the dominant strain in the fungal phylum, with an average relative abundance of 60.2%, followed by Basidiomycota, with an average relative abundance of 16.9% (Figure 2b). The relative abundance of Ascomycota and Mortierellomycota fungi in FP, HYHE, and SHY was significantly higher than that in CK treatment, while the relative abundance of Basidiomycota and Aphelidiomycota fungi in CK treatment was significantly higher than that in other treatments. Relatively speaking, the relative richness of Mortierellomycota fungi treated with FP and the richness of Aphelidiomycota fungi treated with HEHY were significantly higher than those of other treatments.

The distribution of dominant bacterial and fungal species in each treatment at the genus level was evident from the heat map (Figure 3). In the CK, FP, HYHE and SHY treatments, 9, 14, 8 and 12 dominant bacterial species could be identified from the abundant bacterial population; 9, 10, 13 and 15 dominant species could be identified from the fungal population. Cluster analysis showed that there were significant differences in the distribution and relative abundance of dominant bacterial groups among different treatments. The results of studies on fungal genera demonstrated that the distribution of dominant species of fungi treated with HYHE and SHY was similar.

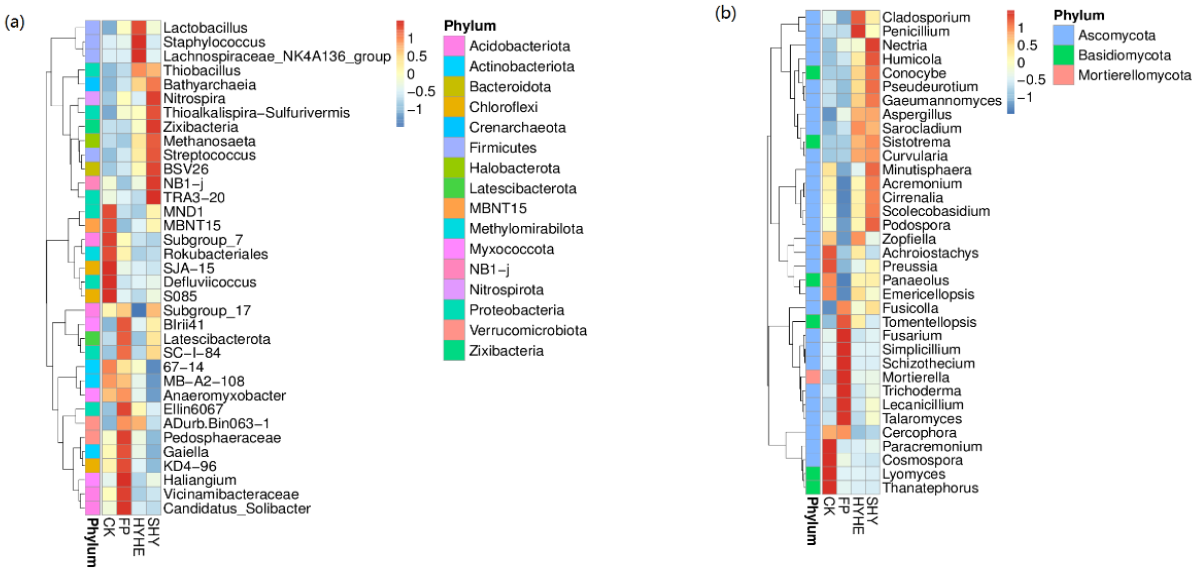

Figure 3. Abundance clustering of bacteria (**a**) and fungi (**b**) at genus level under different management practices.

3.5. Grain Yield

Grain yields for the four different fertilizer treatments during the six-year-long sentinel study were shown in Figure 4, with average annual yields of 6.64 t ha^{-1}, 8.48 t ha^{-1}, 9.59 t ha^{-1} and 10.04 t ha^{-1} for the CK, FP, HYHE, and SHY treatments, respectively. There were significant differences between CK, FP, HYHE, and SHY treatments. Compared with FP treatment, the yields of HYHE and SHY increased significantly, with an average yield increase of 13.1% and 29.0%.

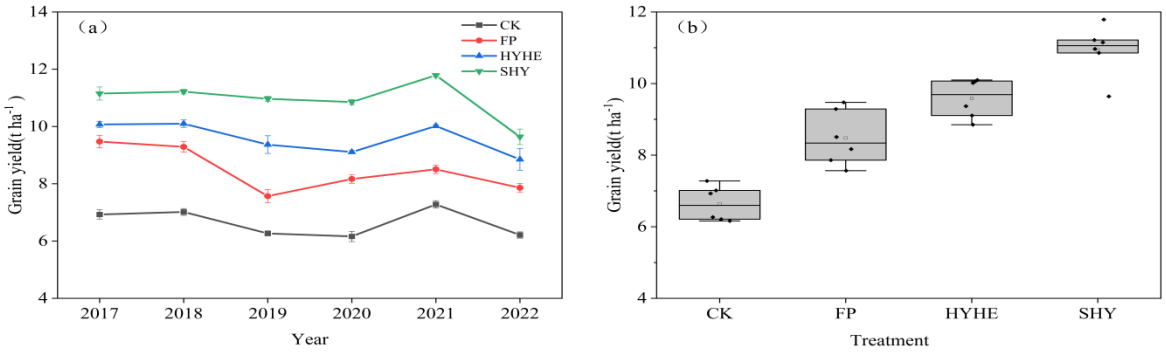

Figure 4. (**a**,**b**) Changes of super hybrid rice yield under different management practices of CK, FP, HYHE, and SHY.

3.6. Correlation of Dominant Microbial Communities with Soil Properties

RDA indicated that axis 1 and axis 2 explain 50.24% and 0.42% of the total variation of soil bacterial community composition, respectively. SOC and NH_4^+-N had significant effects on bacterial communities, explaining 40.3% and 37.2% of the variation (Figure 5a), respectively. In the bacterial phylum, Proteobacteria, Gemmatimonadota, Desulfobacterota, MBNT15, and Bacteroidota were positively correlated with soil TP, AK, AP, NH_4^+-N, and C/N ratio, while Chloroflexi and Actinobacteriota were positively correlated with TN. RDA showed that axis 1 and axis 2 explained 79.94% and 0.19% of the total variation of soil fungal community composition, respectively. A significant effect of AP on fungal communities explained up to 55.9% of the variation. In the fungal phylum, Ascomycota and Mortierellomycota were positively correlated with soil AP, and Basidiomycota was positively correlated with AK and NO_3^--N (Figure 5b).

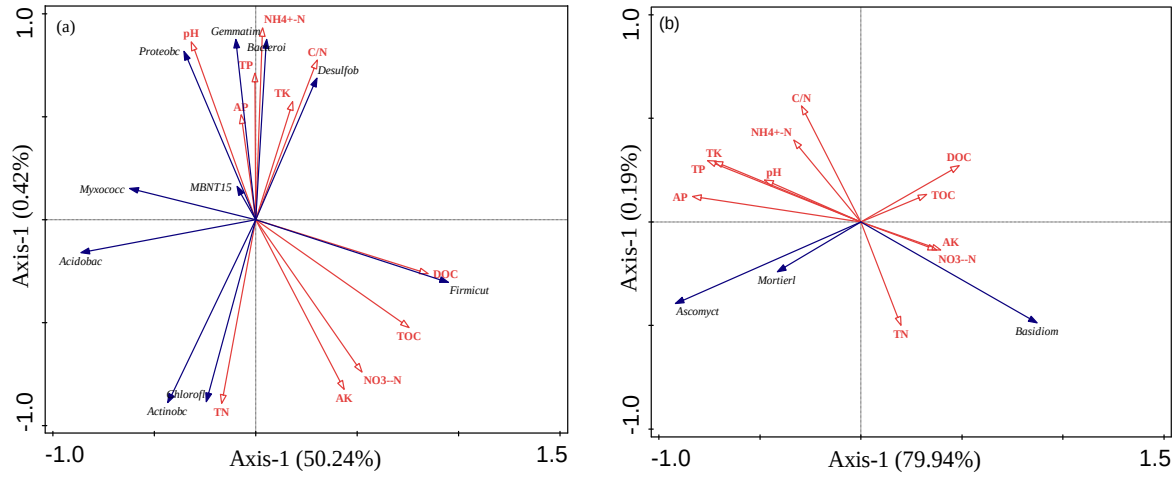

Figure 5. Redundancy analysis of soil physical and chemical properties and main soil bacterial (**a**) and fungal (**b**) communities at the gate level. The red lines represent the physical and chemical properties of the soil, and the blue lines represent the phylum classification of bacteria and fungi.

3.7. Effects of Environmental Factors on Soil Microorganisms and Rice Yield

The relationships among soil physical and chemical properties, bacterial diversity, bacterial community, fungal diversity, fungal community, and yield were studied via PLS-PM analysis ($0.43 < GOF < 0.84$). Soil physical and chemical properties have a direct positive effect on bacterial diversity (path coefficient = 0.80), bacterial community structure (path coefficient = 0.91), fungal diversity (path coefficient = 0.77), fungal community structure (path coefficient = 0.95) and yield (path coefficient = 0.89). Bacterial diversity (path coefficient = 0.89), fungal diversity (path coefficient = 0.58) and fungal community structure (path coefficient = 0.83) had a strong direct positive effect on yield, while bacterial community structure (path coefficient = −0.85) had an indirect negative effect on grain yield (Figure 6).

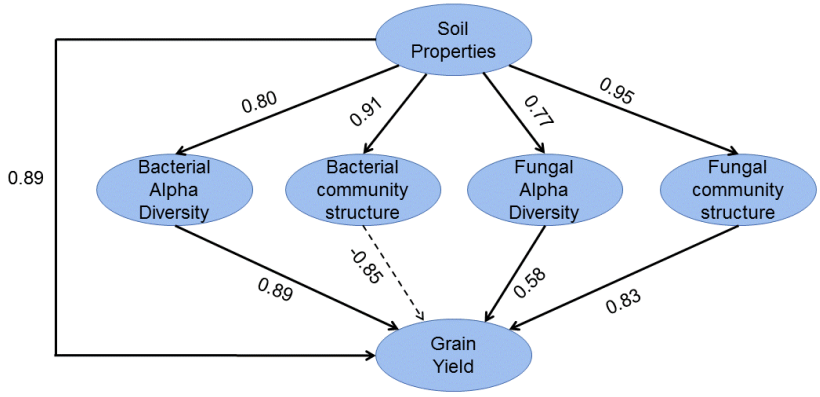

Figure 6. Partial least squares path model (PLS-PM) for rice yield. Lines with arrows represent causality supplemented by path coefficients, while solid lines and dotted lines indicate positive and negative correlations, respectively.

4. Discussion

Improvement in crop yield largely depends on the amount of fertilizer applied; within a certain range, as the nitrogen content increases, rice yield, rice quality and nitrogen fertilizer uptake tend to increase [19,20]. As a result of the study, similar results were found in a six-year field experiment. In four different management practices, with the optimization of fertilizer application rate and fertilizer ratio, the yield of SHY treatment was significantly higher than that of other treatments, while the yield decreased significantly due to extreme high temperatures in 2022. PLS-PM analysis showed that soil physical and

chemical properties and soil microorganisms had a significant direct effect on crop yield, suggesting that crop yield could be increased by enhancing soil microbial diversity and community stability.

Fertility of soil is the most intuitive characteristic of soil health, and soil fertility is an important guarantee for the development of sustainable agriculture. Some studies have shown that soil TN, TK, TP, AP, and SOC increased under long-term balanced fertilization [21]. In this study, after six years of experiment, it was found that the NH_4^+-N, TK, TP, and AP of FP, HYHE, and SHY treatments were higher than those of the CK treatment, and the effect of the SHY treatment was the best, which increased the soil nutrients to increase the yield of rice. Some scholars believe that the use of chemical phosphate or potassium fertilizer decreases soil TN [22]. In this study, it was found that TN and NO_3^--N content decreased in the FP, HYHE, and SHY treatments, which may be due to the increase in grain in the SHY treatment. It has been shown that the use of phosphorus fertilizer decreases significantly with the increase in the amount of phosphorus fertilizer, which is used at a rate of 15% during cultivation, with the rest remaining in the soil [23]. In the present study, it was found that the highest TP and AP contents were found in the soils of the SHY treatment, which is in agreement with the results of previous studies. Some studies have found that excessive application of potassium fertilizer in soil will affect the absorption of Ca^{2+} and Mg^{2+} by plants, cause soil colloid to condense, and lead to soil consolidation, thus reducing soil quality [24,25]. In this study, the input of potassium and phosphorus fertilizer in SHY treatment was higher than that in other patterns, and the contents of TK, TP, and AK in soil were the highest. However, excessive content of P and K would cause a host of soil health problems, such as soil eutrophication and soil consolidation, so optimizing fertilization ratio is the key to maintain soil environment healthy [26,27]. In this study, the pH of soil did not decrease with increased N fertilizer application in the super hybrid rice field, probably due to the combined effect of appropriate fertilizer ratios, application of Zn and Si micronutrient fertilizers, and precise irrigation, which improved N fertilizer utilization and prevented the negative effects of soil acidification caused by N fertilizer enrichment.

Microbial diversity indices were used to reflect species richness and taxonomic proportions across biomes [28]. It was found that the submerged environment would reduce the proportion of fungi and lead to significant changes in the diversity of microbial community structure [29,30]. In our study, it was found that the fungal diversity of HYHE and SHY treatments was significantly higher than that of CK and FP treatments, indicating that the fungal diversity was higher than that of the submerged treatments under alternating dry and wet irrigation. It has been shown that fertilizer application changes bacterial diversity and that different levels of fertilizer application do not have a consistent effect on the diversity of soil microorganisms [31]. This study found that with increased fertilizer levels under different management practices, the bacterial diversity and richness of other treatments were significantly higher than those of the CK treatment [32]. Increased fungal diversity and abundance was more apparent in the HYHE and SHY treatments than FP. This is due to the fact that under different management practices, appropriate fertilizer rates can effectively regulate the microbial environment and indirectly influence plant growth and development and residual yields.

In this study, the analysis of NMDS showed that there were significant differences in bacterial community structure between the four treatments under different management practices and significant differences in fungal community structure between the CK, FP, HYHE, and SHY treatments. Although six years of fertilization application caused differences of soil nutrient content, the effect on the dominant bacteria in each treatment was not significant. Some studies have shown that Proteobacteria, Actinobacteriota, and Chloroflexi have strong activity in soil bacteria. Proteobacteria are currently recognized as the most abundant bacteria in the world [33,34]. Previous studies have found that Proteobacteria, Actinobacteriota, and Acidobacteriota were the three groups with the highest relative abundance in soil bacteria under different fertilization treatments under soybean

continuous cropping [35]. In this study, the relative abundance of Proteobacteria phylum was significantly higher in SHY than that in other treatments. The relative abundance of Actinomycetes and Acidobacteria was higher in the FP treatment than in the other treatments, the relative abundance of Green Curvularia was higher in the CK treatment than in the other treatments, and the relative abundance of Actinomycetes and Green Curvularia was significantly and positively correlated with soil TN. The results show that different management practices changed the physical and chemical properties of the soil, and the increase in fertilizer application increased the amount of carbon and nitrogen in the soil, which provided rich nutrients for Proteobacteria, and the lack of potassium fertilizer stimulated the reproduction of Acidobacteriota bacilli. Therefore, the bacterial community is different under different management practices.

Among the horizontal groups of fungi, Ascomycota, Mortierellomycota, and Basidiomycota are the dominant bacteria in soil, and represent important decomposers of organic substrates such as wood, leaf litter, and manure [36,37]. Previous studies have shown that in Ascomycota Chaetomium is a fungus that breaks down cellulose. It can decompose cellulase and xylanase, which play an important role in the carbon cycle of natural ecosystem and improve soil [38,39]. The dominant phylum communities at the fungal level in this study were found to be Ascomycota and Basidiomycota, with average relative abundances of 60.1% and 16.9%. Compared with CK, other treatments significantly increased the abundance of Ascomycota and decreased the abundance of Basidiomycota, indicating that N and C increased the relative abundance of eutrophic fungi, while decreased the relative abundance of oligotrophic fungi. In addition, the relative abundance of Mortierellomycota was higher in all the FP treatments than in the other treatments. Some of Mortierellomycota species belonged to pathogenic fungi that were susceptible to plant diseases, which is consistent with the results of previous studies [40]. In this study, through cluster analysis at the genus level, it was found that there were significant differences in fungal communities among different management practices, which may be closely related to soil water regulation and nutrient absorption.

Soil microbial species and populations are sensitive to changing environmental factors and anthropogenic disturbance and can reflect changes in the soil in a timely manner [8]. The community structure and diversity of soil microorganisms are the result of the joint action of many soils environmental factors. The study showed that by comparing the physical and chemical properties of soil and microbial community, the contents of TN, NH_4^+-N, SOC, and AP were found to be important parameters affecting microbial community [41]. The results of the RDA analysis showed that soil SOC, TP, NH_4^+-N, and C/N were the main environmental factors affecting bacterial community, while AP and NO_3^--N were the main environmental factors affecting fungal community. Thus, by using appropriate cropping patterns, we can adjust the ratio of soil nutrients, promote the growth of the microbial community involved in carbon and nitrogen cycling, reduce the community abundance of harmful fungi, and increase the grain yield of super hybrid rice.

5. Conclusions

In this study, we have found that management practices, specifically the HYHE and SHY treatments, led to a significant increase in the yield of super hybrid rice. Additionally, these treatments induced notable changes in the soil's physical and chemical properties, including alterations in NH_4^+-N, total P, available P, organic carbon, and available K content. Furthermore, these changes affected the diversity and community structure of soil bacteria and fungi. The bacterial community showed a significant increase in the relative abundance of Proteobacteria with higher fertilizer application, while Actinobacteriota and Chloroflexi exhibited decreased abundance. As for the fungal groups, there was an increase in the relative abundance of ascomycetes and a decrease in basidiomycetes. Notably, the composition of the soil bacterial community demonstrated a close association with organic carbon and NH_4^+-N, while the available P exhibited a significant relationship with the fungal community. These results highlight the positive impact of implementing

reasonable management practices on the soil's physical and chemical properties in super hybrid rice production. Moreover, such practices improve the diversity and stability of the microbial community, ultimately leading to increased grain yield and ensuring the sustainable development of agriculture.

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