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Effects of Long-Term Straw Management and Potassium Fertilization on Crop Yield, Soil Properties, and Microbial Community in a Rice–Oilseed Rape Rotation

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Abstract: The present study aims to assess the influences of long-term crop straw returning and recommended potassium fertilization on the dynamic change in rice and oilseed rape yield, soil properties, bacterial and fungal alpha diversity, and community composition in a rice–oilseed rape system. A long-term (2011–2020) field experiment was carried out in a selected paddy soil farmland in Jianghan Plain, central China. There were four treatments with three replications: NP, NPK, NPS, and NPKS, where nitrogen (N), phosphate (P), potassium (K), and (S) denote N fertilizer, P fertilizer, K fertilizer, and crop straw, respectively. Results showed that long-term K fertilization and crop straw returning could increase the crop yield at varying degrees for ten years. Compared with the NP treatment, the long-term crop straw incorporation with K fertilizer (NPKS treatment) was found to have the best effect, and the yield rates increased by 23.0% and 20.5% for rice and oilseed rape, respectively. The application of NPK fertilizer for ten years decreased the bacterial and fungal alpha diversity and the relative abundance of dominant bacterial and fungal taxa, whereas continuous straw incorporation had a contradictory effect. NPKS treatment significantly increased the relative abundance of some copiotrophic bacteria (Firmicutes, Gemmatimonadetes, and Proteobacteria) and fungi (Ascomycota). Available K, soil organic matter, dissolved organic carbon, and easily oxidized organic carbon were closely related to alterations in the composition of the dominant bacterial community; easily oxidized organic carbon, dissolved organic carbon, and slowly available K were significantly correlated with the fungal community. We conclude that long-term crop straw returning to the field accompanied with K fertilizer should be employed in rice-growing regions to achieve not only higher crop yield but also the increase in soil active organic carbon and available K content and the improvement of the biological quality of farmland.

Keywords: straw management; potassium fertilizer; rice–oilseed rape rotation; yield; bacterial community; fungal community

Citation: Li, J.; Gan, G.; Chen, X.; Zou, J. Effects of Long-Term Straw Management and Potassium Fertilization on Crop Yield, Soil Properties, and Microbial Community in a Rice–Oilseed Rape Rotation. *Agriculture* **2021**, *11*, 1233. https://doi.org/10.3390/agriculture11121233

Academic Editors: Chengfang Li and Lijin Guo

Received: 25 October 2021
Accepted: 4 December 2021
Published: 7 December 2021

1. Introduction

Crop residue is a considerable renewable resource with abundant organic carbon (C) and mineral nutrients [1,2]. As the country with the largest agricultural production in the world, China produces more than 800 million tons of crop straw per year, which amounts to 3.64, 0.73, and 14.78 million tons of nitrogen (N), phosphorus (P), and potassium (K), respectively [3,4]. Straw incorporation serves as the most effective way of comprehensive straw utilization compared with other ways (as burning, compost, or cooking) at present. Many research studies have confirmed that crop residue recycling could increase crop yield

and maintain soil fertility [5–7]. With the increased awareness of environmental protection and ban on burning straw, directly returning straw to the field is being accepted by more and more farmers in China.

Potassium is one of the most essential mineral nutrients for plant growth and metabolism [8]. Adequate soil K supply is beneficial for agricultural production [4]. Because of the promotion of high-yield varieties and high inputs of N and P fertilizer, K deficiency or soil K imbalance has become more widespread and critical in China, especially in the southern multi-cropping region. Paddy-upland rotation, as the main crop rotation system in the southern part of China, is mainly distributed in the rice cropping areas of the Yangtze River Basin and Huang-Huai River Basin [9,10]. However, long-term intensive cultivation removes 210–360 kg ha^{-1} of K_2O per year by crop harvest and has resulted in a substantial decrease in soil available K content [11]. In addition, potash reserves are penurious and expensive in East and South Asia [12]. As a result, farmers have employed less K fertilizer in production. Therefore, the current input of K fertilizer falls short of maintaining the soil K balance, and straw returning is indispensable to improve the K status of cropland [4,11].

Previous studies have showed that crop straw returning could improve soil available K and slowly available K content. Moreover, crop straw incorporation with K fertilizer significantly improved crop yield and maintained soil health [3,10,13]. However, the decomposition rate of straw returning to the field was significantly affected by soil water content. Compared with the upland cropping rotation, the paddy-upland rotation was found to lead to seasonal dry–wet alternation in the farmland system [14,15]. The strong conversion of hydrothermal conditions is bound to affect the decomposition rate of returned straw and the release of straw nutrients, affecting the growth of crops and their absorption and utilization of soil mineral nutrients [16]. Therefore, it is unclear how upland and paddy crops respond to the long-term combinations of K fertilizer with crop residue incorporation.

In addition, crop straw contains abundant organic ingredients, and has been widely applied in fields to promote soil C sequestration [17,18]. Soil microorganisms, such as bacteria and fungi, are the basis of soil fertility and have a great influence on plant health and growth [19,20]. Previous studies have documented the positive and significant impact of straw utilization on soil bacterial and fungal community structure under short-term or long-term straw returning [18,21,22]. However, other research has indicated that straw incorporation decreased the richness and diversity of bacterial and fungal composition. Ling et al. [23] found that the increase in the amount of soil microorganisms after long-term wheat straw returning was mainly due to the increase in the multiplication of bacteria. Additionally, some studies have shown that fertilization could alter the nutrient content of soil (i.e., total N, available P, and available K) and directly drive the evolution of soil microbial communities [24–26]. Wasaki et al. [27] found that the decrease in soil pH, caused by the application of N, was the primary cause of bacterial community changes, and soil C:P and N:P changes determine the composition of the soil microbial communities. Long-term P fertilization increased soil microbial P immobilization by decreasing the relative abundance of the P-starvation response gene and increasing that of the low-affinity inorganic-P transporter gene [28]. In black soil, the alpha diversity and the relative abundance of Acidobacteria significantly decreased with the increased rate of K fertilizer in short-term treatment [29]. Compared with the application of N and P fertilization, K fertilizer has not gained enough attention in soil microbial diversity and composition; especially, the long-term effect of straw incorporation and K fertilizers on bacterial and fungal communities in paddy soils has not been addressed. A recent study showed that the keystone taxa had higher gene copies of oxidoreductase and 71 essential functional genes associated with C, N, P, and sulfur cycling in controlling soil function and wheat production. Meanwhile, the microbial community was highly responsive to K fertilization, which was associated with lower crop production and higher abundance of potential fungal pathogens [30]. In short-term experiments, the yield-increasing effect of K fertilizer was higher than that of straw management [13,16,21]. However, in long-term experiments, the impact of bacterial and fungal community and structure on crop yield is unclear [10,16]. We

hypothesized that long-term K fertilizer application would reduce soil microbial diversity and species composition, and straw returning could mitigate the toxic effects of K fertilizer on microorganisms. Therefore, this study aimed to characterize the dynamics of paddy-upland rotation yield, soil properties, microbial diversity, and community composition in the same site treated with different field management and their associated soil properties, which will provide scientific data of the long-term potential effects to compare against those of short-term field experiments.

2. Materials and Methods

2.1. Experimental Site Description

The field experimental site established in 2011 was located in the town of Chuandian, Jingzhou (part of the Jianghan Plain), Hubei Province, central China (30°33′25″ N, 112°4′53″ E, altitude 80 m). A rice–oilseed rape rotation system was implemented in 1999. The average annual rainfall was 1140 mm, and the air temperature was 15 °C. Soil type was classified as silty clay loam using the World Soil Classification of the Food and Agriculture Organization (sand, 3.5%; silt, 61.0%; clay, 35.5%). At the beginning of the experiment (June 2011), the selected soil basial properties at a depth of 0–20 cm were as follows: pH, 5.97; organic matter, 26.9 g kg^{-1}; total N, 0.61 g kg^{-1}; Olsen-P, 8.1 mg kg^{-1}; available K, 164.8 mg kg^{-1}; slowly available K, 405.4 mg kg^{-1}.

2.2. Experimental Design

A complete randomized block design was conducted with four treatments and three replications. The treatments were (1) NP, chemical fertilizer N, and P application; (2) NPK, balanced chemical fertilizer N, P, and K application; (3) NPS, application of chemical fertilizer N, P plus straw returning, where S represents crop straw; and (4) NPKS, application of chemical fertilizer N, P, K plus straw returning. The dimensions of the plot were 20 m^2, with a length of 5.0 m and a width of 4.0 m. The cropping sequence was rice followed by winter oilseed rape. Rice was transplanted at the age of five leaves at a density of 22 hills m^{-2} (row spacing: 25 cm × 18 cm) and two plants per hill in mid-June and harvested in mid-September. Winter oilseed rape was directly seeded onto the soil surface at a rate of roughly 7.5 kg ha^{-1} in early October, and the crop was harvested in early May. The water regimes were early flooding-mid season drainage intermittent irrigation for the rice season and a rain-fed agricultural regime for the oilseed rape season.

The amounts of N, P, and K fertilizer application for rice and oilseed rape under different treatments are described in Table 1. During the rice season, N (urea, 46% N) was applied in three splits: 60% as basal fertilizer before rice transplanting, 20% at tillering stage, and 20% at the booting stage. K (as potassium chloride, 60% K_2O) was applied as 60% basal fertilizer and 40% booting fertilizer. P (as superphosphate, 12% P_2O_5) was applied manually as basal fertilizers. During the winter oilseed rape season, N was applied in three splits: 60% as basal fertilizer, 20% during the overwintering stage, and 20% at the beginning of stem elongation. P, K, and B (as sodium borate, 11% B) fertilizers were applied manually as basal fertilizers.

In order to ensure the consistency of the experiment, the straw amount of the first crop season (rice) returned to field in 2011 was 2250 kg ha^{-1} of winter rape stalks and shells. The K contents of the stalk and shell were 1.82% and 2.56%, respectively. All straw should be protected from rainfall before returning to prevent K^+ leaching. In the rice season, the oilseed rape straw was crushed by a machine (to a length of 10 cm) and incorporated into the plough layer together with basal fertilizer. In the oilseed rape season, the rice straw was placed as mulch onto the soil with no tillage.

Table 1. The application rates of N, P, K, and B for treatments in both seasons per year from 2011 to 2020 (kg ha^{-1}).

Treatment	Rice Season		Oilseed Rape Season		
	Chemical Fertilizer (N-P_2O_5-K_2O)	Crop Straw (N-P_2O_5-K_2O)	Chemical Fertilizer (N-P_2O_5-K_2O)	Crop Straw (N-P_2O_5-K_2O)	Boron ($Na_2B_4O_7 \cdot 5H_2O$)
NP	180-60-0	0-0-0	180-60-0	0-0-0	15.0
NPK	180-60-90	0-0-0	180-60-90	0-0-0	15.0
NPS	180-60-0	19.4-3.1-142.6	180-60-0	53.4-7.8-164.3	15.0
NPKS	180-60-90	20.2-3.5-151.5	180-60-90	61.6-8.5-179.1	15.0

Note: the nutrient (N, P, and K) apparent input of crop straw was the average value from 2011 to 2020. The conversion coefficients of N, P, and K to N, P_2O_5, and K_2O were 1, 2.3, and 1.2, respectively.

2.3. Sample Sampling and Determination

2.3.1. Grain Yield

For each crop season, the mature oilseed rape and rice plants were harvested and thrashed in each plot, and the grains were dried to determine the grain yield. The crop straw was moved out or fully returned to the field according to each treatment. Before the harvest, five plants of rice and oilseed rape were randomly collected for element analysis of N, P, and K. The sampled plants were partitioned into straw and grain. The dry matter was digested in 70% concentrated H_2SO_4 and 30% H_2O_2 to determine the N, P, and K content in grain and plants.

2.3.2. Soil Samples

On 10 September 2020 (after rice harvest), soil samples were randomly collected from four points in each plot at a depth of 0–20 cm using an auger with a diameter of 5.0 cm. Soil from the four core samples of a plot was mixed to obtain one composite sample. After removing stones, roots, and plant residue using a 2 mm mesh, each sample was divided in half: one half was air-dried for physicochemical analyses, and the other half was immediately stored at −80 °C for soil DNA extraction [22].

2.3.3. 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 (PHS-3C, INESA Scientific Instrument Co. Ltd., Shanghai, China). Olsen-P was extracted with 50 mL of 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) and determined using an injection pump analyzer (AA3, Bran+ Luebbe, Norderstedt, Germany). Available K and slowly available K were extracted with 1 mol L^{-1} NH_4OAc and 1 mol L^{-1} HNO_3 solution, respectively, and measured by a photoelectric flame photometer [24]. The SOM was determined using a wet oxidation procedure with potassium dichromate ($K_2Cr_2O_7$)-sulfuric acid (H_2SO_4). EOC content was measured in 15 mg of each soil sample to which 25 mL of 333 mM $KMnO_4$ was added. Afterwards, the samples were shaken at 200 rpm for 1 h and then centrifuged at 4000 rpm for 5 min. Then, the supernatant was removed and diluted 1:250 with distilled water. The absorbance of the diluted solution was measured at 565 nm. DOC was measured by adding 60 mL of distilled water to 20 g of fresh soil (3:1, v/w) in a 150 mL polypropylene bottle. The samples were shaken on a shaker for 30 min at 250 rpm and then centrifuged for 10 min at 10,000 rpm. The upper suspension was filtered through a 0.45 μm filter into a bottle, and the C content in the filtered solution was determined using a C/N element analyzer (Velp, Usmate Velate, Italy) [10]. Furthermore, soil available N content was determined by the alkaline hydrolysis diffusion method.

2.3.4. Soil DNA Extraction and High-Throughput Sequencing Analysis

DNA was extracted from the soil samples (0.5 g) using a Fast DNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) in accordance with the protocol of the manufacturer. The quantity and quality of the DNA extracts were determined using a NanoDrop

2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The extracted DNA was stored at −20 °C for further analysis.

An aliquot of the extracted DNA from each sample was used as the template for amplification. The V3–V4 hypervariable regions of the bacterial 16S rRNA gene sequences and the ITS region of the fungal rRNA gene sequences were amplified [31]. Amplicon libraries were prepared using tagged bacterial and fungal universal primers, i.e., 338F and 806R for bacteria and ITS1F and ITS2R for fungi. The DNA samples were amplified individually using the fusion primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) for bacteria and ITS1F (5′-CTTGGTCATTTAGGAAGTAA-3′) and ITS2R (5 '-GCTGCGTTCTTCATCGATGC-3′) for fungi to generate polymerase chain reaction (PCR) fragments [32]. The following thermal program was used for amplification: initial denaturation 98 °C for 2 min, followed by 27 cycles of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The PCR reactions were performed in a 25 µL mixture containing 5 µL of 5× reaction buffer, 5 µL of 5×GC buffer, 2 µL of dNTP (2.5 mM), 1 µL of forward primer (10 uM), 1 µL of reverse primer (10 uM), 2 µL of DNA template, 8.75 µL of ddH2O, and 0.25 µL of Q5 DNA polymerase [24]. The PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, San Francisco, CA, USA) and quantified using a Quantus Fluorometer (Promega, Madison, WI, USA). The target sequences were performed on an Illumina MiSeq 250 sequencing platform by Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

2.3.5. Sequence Processing

In sequencing the original data to remove the primer adapter sequence, the processed low-quality bases (maximum expected error higher than 1 for bacteria and 0.5 for fungi, shorter than 370 bp for bacteria and 200 bp for fungi) were removed from downstream analysis [31]. Then, the remaining data were spliced to obtain valid sequence data for each sample. Finally, using 97% as the threshold, the 16S and ITS sequences were divided into operational taxonomic units (OTUs). Using QIIME 2 software, the UCLUST sequence comparison tool was used to cluster with 97% sequence similarity. Each sequence with the highest OTU degree was selected as the representative sequence of the OTU [33]. For bacterial 16S rRNA and fungal ITS genes, both the Greengenes database and the Silva database were used as template sequences for OTU classification status identification [34]. After quality filtering and removal of chimeric sequences, 257,956 and 333,433 high-quality sequences were clustered into 14,906 and 1627 OTUs, respectively, for each bacterial and fungal sample.

2.4. Statistical Analysis

The analysis of variance procedure in SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used to perform data analysis on soil biogeochemical properties and alpha diversity. Before statistical analysis, we tested the normality of the data using the Shapiro–Wilk test. The Shannon and Simpson indexes, abundance-based coverage estimator (ACE), and Chao1 were calculated to estimate alpha diversity of each treatment using MOTHUR [35]. The yield, soil properties, and alpha diversity were tested by one-way analysis of variance (ANOVA), with Duncan's test, at a p value < 0.05. A two-way analysis of variance (ANOVA) was also used to examine the contribution of treatment (T) and year (Y) to crop yield. To determine the structural differences between bacterial and fungal communities at different treatments, an analysis of similarities was also conducted using QIIME2 based on Bray–Curtis distance measurements and abundance data. To determine which taxa were significantly affected, the linear discriminant analysis effect size (LEfSe) algorithm was implemented [17]. The "vegan" package in R language was used to perform similarity analysis. The clustering analysis was constructed using the "heatmap" package based on the Spearman correlation matrix. Each column in the heatmap represents one treatment, and each row represents a genus. The color from red to blue indicates that the abundance is from high to low. Redundancy analysis was used to access the effects of soil environmental

factors on bacterial and fungal communities. To reveal how the potential pathways (soil properties, alpha diversity, and microbial community) influence rice yield and oilseed rape yield, partial least squares path models (PLS_PM) were evaluated using the Goodness of Fit (GOF) statistic [36], and assembled by the "inner plot" function using the "plspm" package of R 4.1.0.

3. Results

3.1. Grain Yield

Over the ten-year study period, grain yield was affected by straw returning, K fertilizer, and planting duration for rice and oilseed rape (Figure 1). The grain yields without K fertilizer (NP treatment) were 9.4 t ha^{-1} and 1.75 t ha^{-1} annual average for rice and oilseed rape, respectively. In the first crop rotation, when the crop straw returned to the field or K fertilizer was applied, the rice yield did not show a significant increase compared with that of NP treatment, but after two rotation cycles, a significant increase could be seen in Figure 1. For the subsequent crop oilseed rape, a yield increase effect appeared in the first rotation. Compared with the NP treatment, the average annual increments of rice and oilseed rape by NPK treatment were 1.5 t ha^{-1} and 0.13 t ha^{-1}, and the average increase rates were 15.8% and 7.4%, respectively. In straw returning (NPS treatment) compared with NPK treatment, the yield increase rate of oilseed rape was higher, while the yield increase rate of rice was the opposite. The yields of rice and oilseed rape for NPKS treatment were the highest, reaching an annual average of 11.6 t ha^{-1} and 2.11 t ha^{-1}, respectively, and the corresponding yield increase rates were 23.0% and 20.5%, respectively.

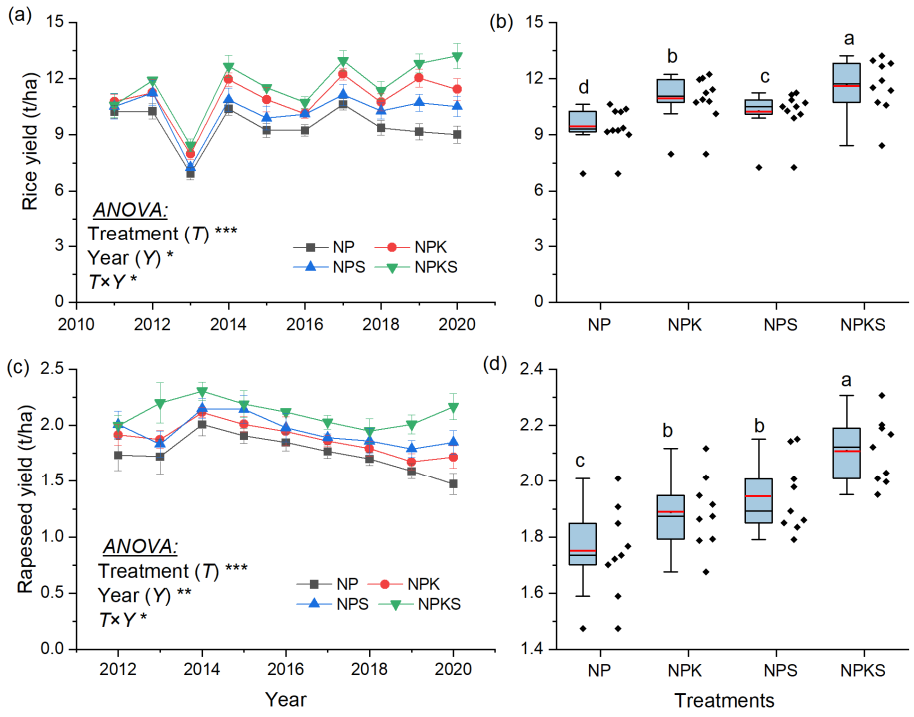

Figure 1. Variation and distribution of grain yields for rice (**a**,**b**) and oilseed rape (**c**,**d**) under different fertilization treatments NP, NPK, NPS, and NPKS over 10 years. *, **, and *** indicate significant differences at the $p < 0.05$, $p < 0.01$, and $p < 0.001$ level, respectively in (**a**,**c**). The upper, middle, and lower limits of each box represent the 75th, 50th, and 25th percentage for crop yield, respectively. Red lines indicate the mean value, and different lower-case letters indicate significant differences for the mean crop yield between treatments at $p < 0.05$ in (**b**,**d**).

3.2. Soil Properties

Table 2 shows the effects of straw incorporation and K fertilizer on the soil properties. From the results, the SOM, available N, available K, slowly available K, EOC, and DOC

differed significantly among the treatments ($p < 0.05$). SOM in NPKS treatments was significantly greater ($p = 0.036$) than in NP treatment and ranked as NPKS, NPS > NPK, NP. There was no significant difference in soil Olsen-P and pH value among the treatments ($p > 0.05$). Soil available K ranged from 169.5 to 254.7 g kg^{-1}, with the highest concentration of that obtained in the NPKS treatment. Slowly available K was lowest with the NPKS treatment, and EOC, and DOC with the NPKS treatment was significantly greater ($p = 0.024$) than those with the NPK and NPS treatments.

Table 2. Effects of straw incorporation and K fertilizer on soil properties in the bulk soils.

Soil Properties	Treatments			
	NP	NPK	NPS	NPKS
pH	5.82 ± 0.03 a	5.76 ± 0.04 a	5.75 ± 0.03 a	5.73 ± 0.02 a
SOM (g kg^{-1})	29.8 ± 2.9 b	31.5 ± 3.4 b	32.8 ± 2.6 ab	35.7 ± 1.4 a
Available N (mg kg^{-1})	77.3 ± 8.9 b	71.8 ± 8.3 b	89.0 ± 6.3 a	85.7 ± 7.4 a
Olsen-P (mg kg^{-1})	9.6 ± 0.5 b	10.1 ± 0.6 b	11.2 ± 1.1 a	10.4 ± 0.8 b
Available K (mg kg^{-1})	169.5 ± 8.8 d	183.0 ± 7.6 c	208.0 ± 11.4 b	254.7 ± 16.2 a
Slowly available K (mg kg^{-1})	601.5 ± 10.3 b	572.9 ± 14.5 c	632.0 ± 16.7 a	529.0 ± 14.8 d
EOC (g kg^{-1})	5.9 ± 0.5 c	5.1 ± 0.2 c	7.6 ± 0.4 b	9.5 ± 0.3 a
DOC (mg kg^{-1})	20.8 ± 0.4 c	22.0 ± 1.0 c	27.0 ± 0.9 b	30.6 ± 1.2 a

Note: SOM, soil organic matter; EOC, easily oxidized organic carbon; DOC, dissolved organic carbon. Within a row, data (mean ± SD, $n = 3$) followed by different letters are significantly different ($p < 0.05$).

3.3. Alpha Diversity of Bacterial and Fungal Communities

A total of 364,587 and 336,310 filtered sequences remained after quality control, and 257,956 and 333,433 reads (high-quality sequence) were generated for further bioinformatic analysis (Table S1). All these sequences were subsequently clustered into 14,906 and 1627 OTUs based on 97% similarity. The number of observed OTUs detected in each sample ranged from 2890 to 4426 and 298 to 535 for bacterial and fungal groups, respectively. Good's coverage index of each sample was >0.990. The rarefaction curves (Figure S1) were close to the saturation phase, indicating that sufficient sequencing coverage was achieved and that the OTUs were representative of the overall microbial community libraries.

There were significant differences among the treatments in the alpha diversity, except the Simpson index, of bacterial and fungal populations, as shown by richness and diversity indexes (Table 3). Among the treatments, NP treatment had the highest value of Chao1 and ACE, suggesting that long-term non-K fertilizer application resulted in greater richness of bacterial populations than the other treatments. The Shannon index was significantly higher for the NP and NPS treatments than for the NPK and NPKS treatments, but no significant difference was observed between the treatments on the Simpson index. Meanwhile, this tendency was shown in the fungal group that NPK and NPKS treatments had lower richness and diversity indexes than those of NP and NPS treatments.

Table 3. Alpha diversity of bacterial and fungal gene sequences in the soil samples.

Microbe Type	Treatment	Richness Index		Diversity Index		Coverage
		Chao1	ACE	Simpson	Shannon	
Bacteria	NP	4463 ± 142 a	4422 ± 135 a	0.999 ± 0.031 a	10.98 ± 0.06 a	0.994 ± 0.035 a
	NPK	3664 ± 165 b	3470 ± 124 b	0.998 ± 0.045 a	10.27 ± 0.05 b	0.990 ± 0.051 a
	NPS	4298 ± 151 a	4128 ± 128 a	0.998 ± 0.037 a	10.65 ± 0.08 ab	0.998 ± 0.048 a
	NPKS	3045 ± 123 c	2897 ± 135 c	0.993 ± 0.033 a	9.33 ± 0.06 c	0.991 ± 0.044 a
Fungi	NP	535 ± 21 a	518 ± 19 a	0.952 ± 0.031 a	6.45 ± 0.04 a	1.000 ± 0.045 a
	NPK	298 ± 11 c	264 ± 16 d	0.958 ± 0.043 a	5.82 ± 0.03 b	1.000 ± 0.053 a
	NPS	477 ± 18 b	453 ± 21 b	0.953 ± 0.036 a	5.98 ± 0.04 b	1.000 ± 0.039 a
	NPKS	317 ± 16 c	311 ± 14 c	0.966 ± 0.045 a	6.23 ± 0.04 ab	1.000 ± 0.044 a

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

3.4. Composition of Bacterial and Fungal Communities

Long-term straw returning and K fertilizer altered the relative abundance of bacterial and fungal phyla in soil (Figure 2). Proteobacteria and Acidobacteria had the highest relative abundance in each treatment, belonging to the predominant bacterial community (relative abundance > 15.0%), with averages of 32.0% and 16.9%, respectively. The relative abundances of Actinobacteria, Chloroflexi, Nitrospirae, Rokubacteria, Bacteroidetes, and Verrucomicrobia were higher, with averages of 4.3%, 2.5%, 2.1%, 2.0%, 1.4%, and 1.1%, respectively (Figure 2a). In the fungi phylum (Figure 2b), Ascomycota was the dominant species, with an average relative abundance of 48.1%, followed by Basidiomycota and Mortierellomycota with average relative abundances of 24.8% and 4.4%, respectively.

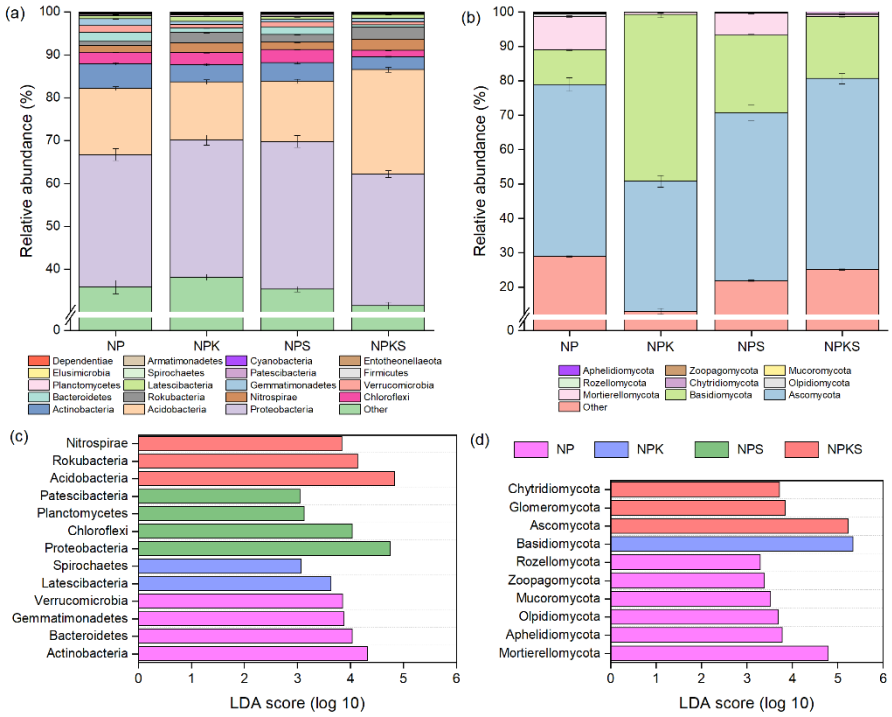

Figure 2. The relative abundance of major taxonomic groups for (**a**) bacteria and (**b**) fungi at the phylum level and the error bars are standard deviation and significant changes in bacterial (**c**) and fungal (**d**) key phylotypes identified using linear discrimination analysis effect size.

At the level of bacterial phylum (Figure 2c), Actinobacteria, Bacteroidetes, Gemmatimonadetes, and Verrucomicrobia were significantly altered taxa in the NP treatment. Latescibacteria and Spirochaetes were found to be sensitive to NPK treatment. Intriguingly, the predominant Proteobacteria, Chloroflexi, Planctomycetes, and Patescibacteria were significantly altered in the NPS treatment, and only Acidobacteria, Rokubacteria, and Nitrospirae were positively altered in the NPKS treatment. At the level of fungal phylum (Figure 2d), Mortierellomycota, Aphelidiomycota, Olpidiomycota, Mucoromycota, Zoopagomycota, and Rozellomycota were the significantly altered taxa in the NP treatment. Basidiomycota was sensitive to NPK treatment. Conversely, there were no taxa changed in the NPS treatment. Ascomycota, Glomeromycota, and Chytridiomycota were clearly altered in the NPKS treatment. These results indicated that long-term application of N and P fertilizer without K fertilizer stimulated an increase in the species and relative abundance of oligotrophic bacteria and fungi. At the same time, the application of straw with K fertilizer contributed to the increase in existing eutrophic microorganisms.

The species composition of bacteria and fungi at the genus level is shown in Figure 3. The results indicate that the relative abundances of dominant species in the fungal community for four treatments were more significant than those of the bacterial community.

At the bacterial genus level (Figure 3a), Anaeromyxobacter was the dominant genus, with an average relative abundance of 1.56%, followed by Haliangium, Nitrospira, Geobacter, Candidatus Solibacter, Candidatus, Udaeobacter, and Sh765B-TzT-35, with average relative abundances of 0.90%, 0.83%, 0.73%, 0.67%, 0.57%, and 0.54%, respectively. Among them, Haliangium, Candidatus Solibacter, and Candidatus Udaeobacter had the highest relative abundance in NP and NPS treatments, and Nitrospira and Sh765B-TzT-35 had the highest relative abundance in NPK and NPKS treatments.

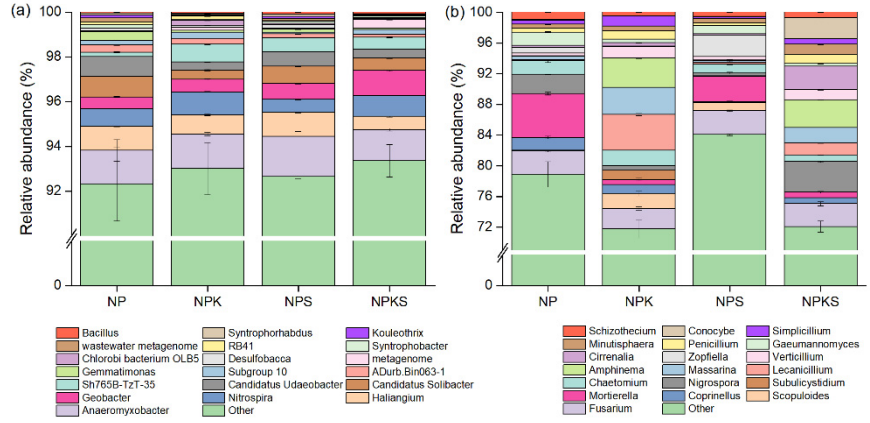

Figure 3. The relative abundance of dominant taxonomic groups for (**a**) bacteria and (**b**) fungi at the genus level. The data represent the mean values of the three replications. Values are means (n = 3); error bars are standard deviation.

At the genus level of fungi (Figure 3b), the dominant species were Fusarium and Mortierella, having average relative abundances of 2.95% and 2.61%, respectively, followed by Nigrospora, Chaetomium, Lecanicillium, Massarina, and Amphinema, with averages of 1.85%, 1.45%, 1.66%, 1.55%, and 1.89%, respectively. The relative abundance of Mortierella was increased significantly in the NP and NPS treatments than in the NPK and NPKS treatments. Among all treatments, Nigrospora (3.94%) in the NPKS treatment had the highest relative abundance, while Chaetomium, Lecanicillium, Massarina, and Amphinema had higher relative abundance in NPK treatment.

3.5. Beta Diversity of Bacterial and Fungal Communities

The Venn diagram (Figure 4a,b) shows that the microbial population had both shared components and unique parts. NPK, NPS, and NPKS treatments shared 8.75%, 11.24%, and 6.31% of the bacterial OTUs with NP treatment, while the unique OTUs of NPK, NPS, NPKS, and NP were 16.36%, 20.88%, 14.09%, and 26.23%, respectively. NPK, NPS, and NPKS shared 5.77%, 9.74%, and 5.68% of fungal OTUs with CK, while the unique OTUs of NPK, NPS, NPKS, and NP treatments were 14.58%, 23.77%, 15.2%, and 27.59%, respectively. These indicate that long-term straw returning and K fertilizer application caused differences in soil microbial communities, thereby affecting the diversity of bacteria and fungi groups among treatments.

A PCoA plot showed that bacterial communities in soils treated with K fertilizer were distinct from those in soils treated with non-K fertilizers along the x-axis (Figure 4c), and the first principal component (x-axis) accounted for 52.6% of the total variation. Still, the straw returning also regulated the communities along the y-axis, but the second principal component (y-axis) only contributed 25.0% of the variation in communities. Similarly, the first two principal coordinates represented 74.7% of the variation in fungi (Figure 4d) communities according to the PCoA, in which the first principal component (x-axis) accounted for 38.1% of the total variation.

From the heatmap (Figure 5), the distribution of dominant bacteria in each treatment was well-marked; specifically, in the NP treatment, up to 18 species could be identified

in the number of abundant bacteria. On the contrary, the abundant bacteria in the NPK, NPS, and NPKS treatment were 12, 14, and 6 types of species, respectively. Furthermore, the cluster analysis results reflected that NP and NPS treatments were similar, and NPK and NPKS were similar. The results of the fungal genus also showed that the distribution and relative abundance of the dominant fungal groups in each treatment were significantly different. Additionally, the cluster analysis indicated that the NP and NPS treatments were homogeneous.

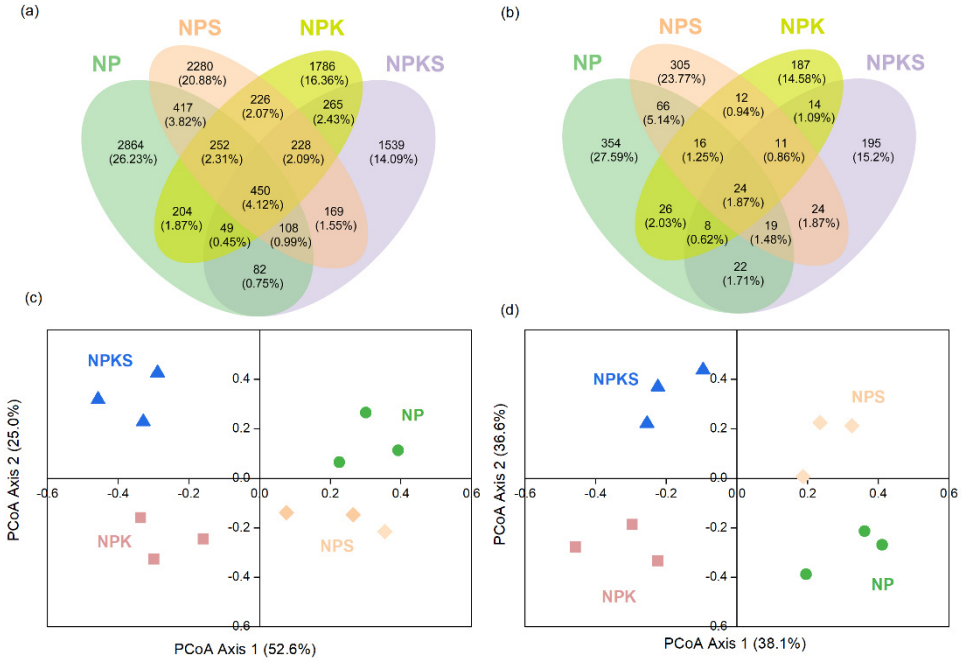

Figure 4. Venn diagram of (**a**) bacterial and (**b**) fungal operational taxonomic units from soil samples and the principal coordinates analysis (PCoA) plot of the dissimilarity between the treatments for (**c**) bacteria and (**d**) fungi based on Bray–Curtis differences.

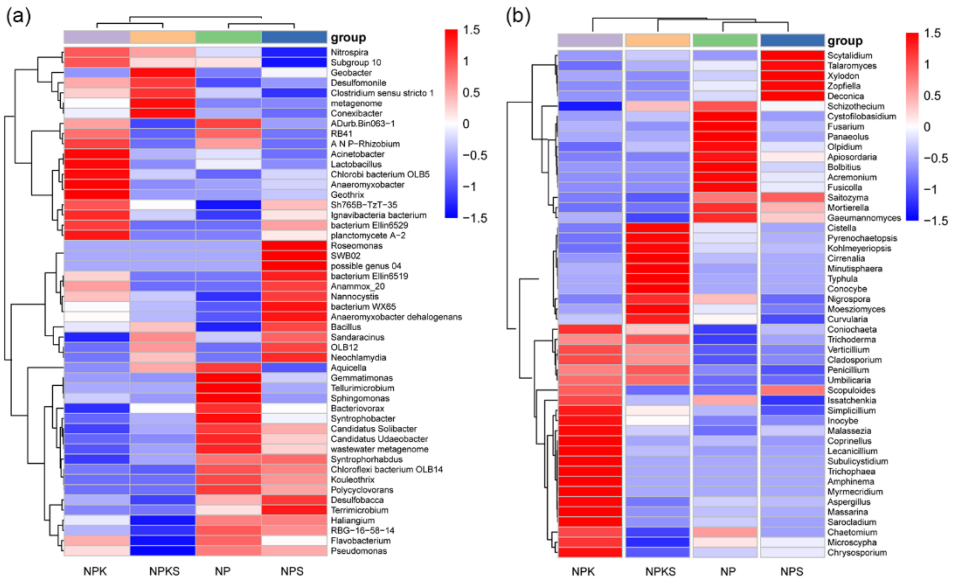

Figure 5. The dominant (**a**) bacteria and (**b**) fungi variances among the treatments at the genus level.

3.6. Correlation of Dominant Microbial Communities with Soil Properties

The redundancy analysis (Pseudo-F = 388, p = 0.002 **) showed that axis 1 and axis 2 explained 80.6% and 14.8% of the total variance in soil bacterial community composition, respectively. The phyla Rokubacteria, Nitrospirae, and Acidobacteria were clustered together to the edge of soil DOC, SOM, available K, and EOC. In contrast, the phyla Chloroflexi, Bacteroidetes, Actinobacteria, and Verrucomicrobia were highly correlated with slowly available K. The available K, SOM, DOC, and EOC had a noteworthy impact on the bacterial community, which explained the variation by 51.3%, 19.8%, 18.8%, and 9.1%, respectively (Figure 6a). The redundancy analysis (Pseudo-F = 518, p = 0.002 **) showed that axis 1 and axis 2 explained 84.6% and 13.9% of the total variance in soil fungal community composition, respectively (Figure 6b). The Ascomycota had a positive correlation with soil available K, DOC, and EOC; the Basidiomycota was highly corrected with slowly available K; and the phylum Mortierellomycota was negatively correlated with slowly available K, available N, pH, and EOC. The EOC, DOC, and slowly available K explained the variation by 27.8%, 57.9%, and 12.5%, respectively.

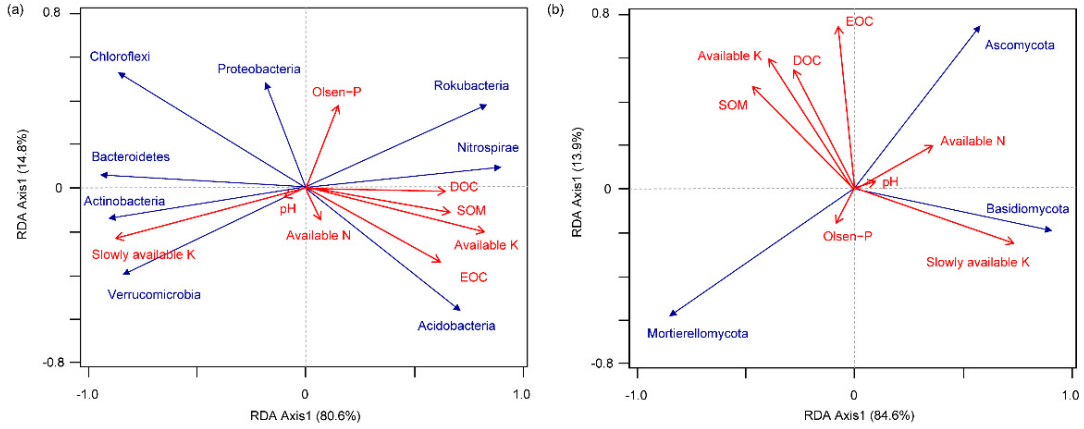

Figure 6. Redundancy analysis of soil properties and main (**a**) bacterial and (**b**) fungal communities at the phylum level in soils. Red lines represent soil properties; blue lines represent the bacterial and fungal phylum−level taxonomy.

3.7. Potential Pathways Influencing Crop Yield

The PLS_PM analysis showed that the final model had GOF of 0.81 and 0.54 for bacterial group and fungal group in 2020, respectively. The pathways of soil properties, alpha diversity, and bacterial community composition together explained 82.3% of the total variance in crop yield, while those represented 45.2% of the variation in fungal group (Figure 7b). The direct effect of soil properties (path coefficient = 0.85) on the crop yield was greater than the direct effect of bacterial community composition (path coefficient = −0.50) and alpha diversity (path coefficient = 0.47) as well as fungal community composition (path coefficient = −0.37) and alpha diversity (path coefficient = 0.31). Moreover, the PLS_PM analysis suggested that soil properties indirectly affected the crop yield by changing bacterial alpha diversity (path coefficient = 0.92) and community composition (path coefficient = −0.88) as well as fungal alpha diversity (path coefficient = 0.66) and community composition (path coefficient = −0.70) in 2020.

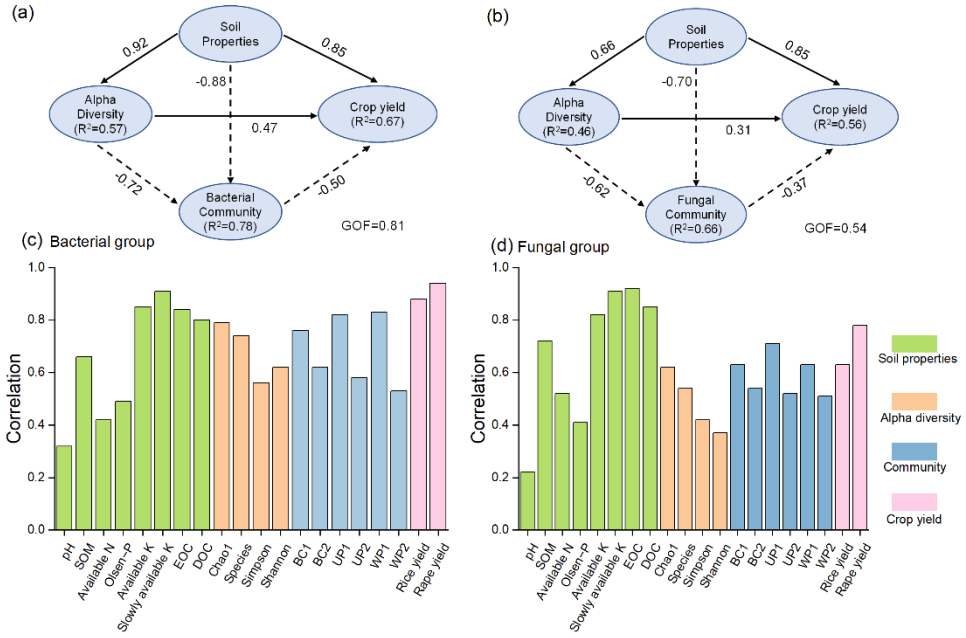

Figure 7. Partial least squares path models (PLS_PM) for the rice and oilseed rape yield in 2020. A line with an arrow indicates a causal relationship, supplemented by a path coefficient, and continuous and dashed lines indicate positive and negative relationships for (**a**,**b**), respectively; the amount of the variability explained by the variables for (**c**,**d**). Path coefficients are calculated after 1000 bootstraps. BC1: Bray−Curtis PCoA1; BC2: Bray−Curtis PCoA2; UP1: Unweighted Unifrac PCoA1; UP2: Unweighted Unifrac PCoA2; WP1: Weighted Unifrac PCoA1; WP2: Weighted Unifrac PCoA2.

4. Discussion

4.1. Effects of Long-Term of Straw Returning and K Fertilizer on Crop Yield

Many studies have reported that straw returning increased crop yields and nutrient uptake [22,37,38]. Our study showed similar results during ten-year field experiments where the same fertilizer inputs were applied among the four treatments, especially for oilseed rape (Figure 1). Through the investigation of yield structure components, the main reason for the increase in crop yield is that straw return significantly improved the number of productive ear and spike granules of rice and wheat, and the number of siliques per plant and the number of seed per pod of oilseed rape [39,40]. In paddy-upland rotation, the yield-increasing effect of the upland season (wheat, oilseed rape) was greater than the rice season. The phenomenon could also be seen in Figure 7, which shows that the soil properties, microbial alpha diversity, and community composition had higher relationships with oilseed rape yield than rice yield and the direct effect of soil properties as SOM, EOC, SOC, and available K content on yield increase were greater than those of the bacterial and fungal groups. Overall, compared with no straw returning, the increase rate of rice yield with straw returning was 5.2%, whereas the yield increase rates of oilseed rape and wheat with straw returning were 10.5% and 12.4%, respectively, in southern China, higher than that of rice [41]. The result of Figure 1 confirmed that the increase rate of oilseed rape in NPS treatment was higher than that of rice. Moreover, as the experiment progressed, the increase rate of yield in field under straw management was more noticeable compared with those with no straw returning. Wang et al. [42] showed that the yield-increasing effect of straw returning was influenced by the annual average temperature, soil nutrient status, returning period, and fertilization. In the study, the increased rate of yield in NPK and NPS treatments did not reach a significant level in the first and second year of rice season, but the third year of rice shows a significant difference compared to that of NP treatment. However, the oilseed rape season showed a significant increase in production during the first crop rotation. This is because the contribution rates of K fertilizer to the rice and oilseed rape were 8.2% and 11.5%, and the dependent rates of soil K status for rice and

oilseed rape were 83.0% and 75.2%, respectively [43]. Therefore, the K element absorbed by the whole rice plant mainly comes from the soil supply, while oilseed rape and wheat are more dependent on exogenous K fertilizer supply.

From the dynamic changes of crop yield over ten years, the increase rate of NPK treatment was slightly better than that of NPS treatment in the rice season compared to NP treatment, and the result was the opposite in the oilseed rape season. Our previous multi-field results also indicated that the yield-increasing rate of K fertilizer application was better than that of straw returning in the rice season. Straw returning involved in not only the process of the release of N, P, and K mineral elements but also the process of straw self-decomposition [44]. High temperature, waterlogging, and straw rot accelerated in rice seasons, which caused a certain toxic effect on root by a higher concentration of phenols and organic acids from straw decomposition, thereby weakening the yield increasing rate of straw returning [45,46]. The decomposition rate of straw in the oilseed rape season was relatively slower, the nutrient release cycle was longer than those of the rice season, and the poison caused by straw rot was relatively weak. In addition to the biochemical effects, the straw mulching in the oilseed rape season has physical effects, such as enhancing crops to resist the resistance of adversity (low temperature and drought) and relieving temperature changes in winter, which was beneficial to crop straw returning [47–49].

4.2. Effects of Long-Term Straw Returning and K Fertilizer on the Soil Properties

In the present study, NP treatment led to the decline tendency on crop yield and lowest chemical properties after 10 years of experimentation. However, straw returning and K fertilizer application significantly improved the soil chemical properties, including the SOM, available K, slowly available K, EOC, and DOC, compared with those of NP treatment; among these treatments, NPKS treatment performed the best (Table 2). However, NPK treatment reduced soil available N content, consistent with the findings of Liu et al. [18] who reported a decline in soil total N with chemical P or K fertilizer. The reason might be that NPK treatment produces more grain than NP treatment resulting in more N uptake and soil N consumption, particularly available N under the same input of chemical N fertilizer. Moreover, the contents of available K, EOC, and DOC were significantly higher in treatments NPKS and NPS than those in NPK and NP treatments, while the slowly available K content showed the opposite trend (Table 2). These results confirmed that the accurate application of K fertilizer rate could not maintain the soil K balance without regard to straw returning in the rice–oilseed rape rotation system [50].

K fertilizer in combination with straw returning could improve the soil available K content [4]. On the one hand, straw returning brings in a large amount of straw K into the farmland; on the other hand, the root secretion of crops and the humification in the process of residual straw rot would weaken the fixation of K^+ on the clay mineral, promoting transformation of slowly available K to water-soluble K and available K, and maintain a new dynamic balance of various soil K forms [51,52]. Notably, the soil EOC and DOC had significant differences between the straw incorporation and without straw. Previous studies demonstrated that straw decomposition facilitated the accumulation of active organic carbon and the release of nutrients in the soil [10]. Therefore, NPKS treatment has been proven to significantly improve soil K and OM content synchronously compared with no straw or K fertilizer input, thereby enhancing crop yield and soil fertility via microbial activities (Figure 7).

4.3. Effects of Long-Term Straw Returning and K Fertilizer on the Soil Microbial Alpha Diversity

As the results show, the microbial community structure and composition are closely related to the soil properties (Figure 7). Different fertilization treatments changed the physical and chemical soil properties of farmland, affecting the community diversity of soil microorganisms. Consistent with the previous results reported in the paddy-upland rotation system [53,54], alpha diversity analysis showed different effects of long-term straw incorporation and fertilization on the richness and diversity of the microbial com-

munity. The PCoA plot showed significant differences ($p < 0.05$) in bacterial and fungal community structure under different treatments (Figure 4), which was consistent with the results of Bai et al. [11], who documented clear separation of bacterial and fungal community composition between the straw utilization with no straw utilization and K fertilizer application with no K fertilizer. In this experiment, compared with NP treatment, the application of chemical K fertilizer (NPK treatment) for ten years caused bacterial and fungal richness index and diversity index to decrease (Table 3). Guo et al. [25] had shown that long-term single fertilizer significantly reduces the richness and diversity of bacteria, which was consistent with our result. However, the addition of crop straw (NPS and NPKS treatment) could increase the richness index and diversity index compared with those of NPK treatment. This was because straw incorporation provided exogenous organic carbon resources for bacteria and fungi living, which was conducive to their breeding growth, reduced competition between them, and enhanced diversity of soil bacterial and fungal communities [17,38]. Therefore, straw incorporation directly affects the microbial alpha diversity by promoting or inhibiting the change of soil bacterial and fungal composition, further impacting the soil biological fertility.

Furthermore, the correlation analysis of soil properties and diversity index (Tables S2 and S3) implied that the Simpson and Shannon indexes of bacteria and fungi were significantly positively correlated with SOM, EOC, and DOC and that they had a negative correlation with pH and available K. Prior studies all have shown that the adverse impact of soil acidification in paddy soil caused by the superfluous application of N or NPK fertilizer on soil microbial diversity far exceeded the positive effect of fertilization [55,56]. Therefore, applying organic fertilizer in combination with NPK chemical fertilizer was reported to be more effective than applying NPK fertilizer alone in the future for microbial diversity.

4.4. Effects of Long-Term Straw Returning and K Fertilizer on the Soil Microbial Community

At present, there has been no more attention as to the effect of long-term straw returning with K fertilizer on soil microbial community composition in paddy soil, and the reported results were not consistent [57]. In this study, the predominant bacterial phyla (Figure 2) in four treatments were Proteobacteria and Acidobacteria, at an average of 32.0% and 16.9%, which is consistent with those reported by Wang et al. [31] and Guo et al. [25] based on agricultural soils. Although the 10-year fertilization results in differences in soil nutrient content, the effect of that on the category of predominant bacteria in each treatment was not noteworthy. Proteobacteria, Nitrospirae, Firmicute, and Actinobacteria (i.e., R-strategist) are considered as copiotrophic groups, while Acidobacteria, Actinobacteria, Chloroflexi, and Planctomycetes were typical oligotrophic (i.e., K-strategist) bacteria [58]. Sun et al. [59] found that the relative abundance of Proteobacteria was significantly positively correlated with soil C and N content. Our study also confirmed that NPS treatment had a higher relative abundance of Proteobacteria than other treatments, while the application of K fertilizer had no significant impact on the dominant bacterial groups (Figure 2). Both long-term and short-term studies have found that the abundance of Acidobacteria decreased significantly with the increase in NPK fertilizer, which was closely associated with soil pH value [56,60], whereas, in our study, the relative abundance of Acidobacteria, serving as typically oligotrophic bacteria in NP (15.4%) and NPKS (24.3%) treatments, was higher than that in NPK (13.6%) and NPS (14.8%) treatments, in contradiction with previous research. This may be related to the increase in the special subgroup function of Acidobacteria, which, in terms of the genus, were unclassified Subgroup 3, unclassified Subgroup 6, unclassified Subgroup 17, and uncultured Desulfovirga sp. (Figure 3). Additionally, the content of available K in NP was not enough for plant growth, and thereby insufficient K application could stimulate the propagation of Acidobacteria phylum community, activating the insoluble mineral ions in the soil.

In the fungi groups, Ascomycota (eutrophic) and Basidiomycota (oligotrophic) were the dominant phyla [11,31] communities in the four treatments, at an average of 48.1% and 24.8%, respectively; they are important decomposers based on organic substrates, such

as wood, fallen leaves, and feces [17]. Figure 2 shows that compared with NP treatment, NPS and NPKS treatments significantly increased the abundance of Ascomycota and significantly decreased the relative abundance of Basidiomycota and Mortierellomycota. In contrast, the NPK treatment had the contrary result, which is in accordance with that reported by Wang et al. [31]. From the result of Table 2, we observed that the NPK treatment had a lower SOM and active carbon content in contrast to the NPS and NPKS treatments. As a class of fungi that can decompose cellulose into Ascomycota, Chaetomium can decompose cellulase and xylanase, which play important roles in the carbon cycle of the natural ecosystem and can result in soil improvement [61,62]. This again proves that abundant organic carbon leads to an increase in the relative abundance of eutrophic fungi and a decrease in the relative abundance of oligotrophic fungi. Moreover, the relative abundance of Mortierellomycota and Olpidiomycota in the NP treatment was higher than that in others. Some of those species belonged to pathogenic fungi generating polyketides, terpenoids, and nonribosomal peptides to cause plant disease [31]; hence, insufficient K content may induce the growth and reproduction of harmful fungi in paddy soil [63]. Particularly, according to the results of the cluster analysis at the genus level, there were significant differences in the distribution of fungal species among nutrient deficiency treatments (Figure 5), which may be closely related to soil nutrient status. This phenomenon was also found in the results of redundancy analysis, i.e., EOC, DOC, and slowly available K had the maximal influence on the fungal community (Figure 6b).

As can be seen from Table 2, after 10 years of fertilization management, the soil organic C resource and the available K content changed significantly among treatments; in particular, the available K content in the NP and NPK treatments decreased significantly, which was mainly because crop harvest took away a large amount of K, leading to the imbalance of K in farmlands [53]. However, straw incorporation could clearly increase active organic C, as EOC and DOC. Therefore, the content of EOC, DOC, available K, and slowly available K became the most important index affecting the relative abundance of microbial community. Fan et al. [63] combined ecological network theory with the ecological resistance index to evaluate the responses of microbial community to wheat production under the condition of long-term fertilization. Their results suggest that the microbial resistance indirectly drives the effects of nutrient fertilization on plant production. Two mechanisms may explain the role of microbial resistance to nutrient fertilization in the promotion of plant production: (1) resistant microbial community with organic fertilizer addition could facilitate plants acquiring more nutrients and less competition from microbial species in soil; (2) low responsive microbial community may lead to lower relative abundance of potential fungal plant pathogens. Our research also indicated that the microbial diversity and community influenced rice yield and oilseed rape yield; moreover, the bacterial community had a higher impact than fungal community on the crop yield. Therefore, crop straw residue returning could alleviate the toxic effect of long-term potash fertilization on microbial population and stimulate crop yield as well as soil C sequestration.

5. Conclusions

In the present study, we found that ten years of continuous crop residue management and K fertilizer application in the rice–oilseed rape rotation significantly improved the crop yield; altered soil physiochemical properties such as SOC, EOC, DOC, available K, and slowly available K; and, therefore, modified the diversity and composition of soil bacterial and fungal communities. The long-term application of K fertilizer and straw returning had a significant increase rate in yield after one crop rotation, and NPKS treatment resulted in the best effect. The application of K fertilizer significantly decreased the richness and diversity index of soil bacterial and fungal populations compared to NP treatment. At the same time, continuous straw incorporation could alleviate the negative effect of K fertilizer on microbial composition. The NPKS treatment increased the relative abundance of the copiotrophic bacteria, such as the Firmicutes, Gemmatimonadetes, and Proteobacteria phyla, and the relative abundance of Ascomycota. Available K, SOM, DOC, and EOC

were closely related to alterations in the composition of the soil bacterial community; EOC, DOC, and slowly available K were significantly correlated with fungal community. These findings provide deep insights into the role of straw incorporation coordinated with K fertilizer on the dynamic change of yield of rice and oilseed rape, and shape soil bacterial and fungal communities and their relationship with soil properties and crop yield.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11121233/s1, Figure S1: Rarefaction curves for (a) bacteria and (b) fungi in the four treatments. Table S1: Detailed sequencing depth results of soil samples under different treatments. Table S2: The correction of soil properties with bacterial community structures. Table S3: The correction of soil properties with fungal community structures.

Author Contributions: Conceptualization, J.L. and J.Z.; methodology, J.L.; software, G.G.; formal analysis, X.C.; investigation, G.G. and X.C.; writing—original draft preparation, J.L.; writing—review and editing, J.L. and J.Z.; funding acquisition, J.L. and G.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported in part by the Open Fund of Key Laboratory of Fertilizer Utilization, Ministry of Agriculture and Rural Affairs, China (KLFAW201901) and the Open-end Fund of Engineering Research Center of Ecology and Agricultural Use of Wetland, Ministry of Education (KF2020015).

Conflicts of Interest: The authors declare no conflict of interest.

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