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Effects of Straw-Return Method for the Maize–Rice Rotation System on Soil Properties and Crop Yields

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Abstract: Exploring suitable maize straw-return measures is essential for the new double-cropping system of maize (*Zea mays* L.)–rice (*Oryza sativa* L.) rotation in the middle reaches of Yangtze River in China, which can increase crop yield by improving soil quality. In this study, four straw-return measures were evaluated by investigating the soil bulk density (BD), organic matter (OM), microbial community, and nutrients from 2016 to 2018. The four straw-return treatments were as follows: (1) no straw-return (CK), (2) only rice straw incorporated into the field (M_0R_i), (3) both maize and rice straw incorporated to field (M_iR_i), and (4) maize straw mulched and rice straw incorporated into the field (M_mR_i). Compared to CK, two-season crop straw-return treatments changed soil microbial community composition, and increased soil total organic carbon (TOC) and dissolved organic carbon (DOC), microbial biomass carbon (MBC), mineralized nitrogen (N_{min}), available phosphorus (P) and exchangeable potassium (K) in the 0–20 cm soil layer by 3.6%, 63.4%, 38.8%, 12.4%, 39.7%, and 21.6%, respectively, averaged across M_mR_i and M_iR_i treatments. In addition, M_mR_i and M_iR_i increased annual yield by 9.1% and 15.2% in 2017 and 11.7% and 12.9% compared to CK in 2018, respectively. M_mR_i exhibited superiority in the soil microbial community, enzyme activities, DOC, MBC, N_{min}, available P, and exchangeable K in contrast to M_iR_i. We concluded that M_mR_i is the best measure to implement for straw-return in maize–rice rotation systems.

Keywords: maize–rice rotation; straw management; soil organic matter; soil microbial community; soil nutrients

1. Introduction

The middle Yangtze River Basin, located in a subtropical monsoon climate zone, has abundant climatic resources and is an important crop production region in China. The main cropping systems in this area are double- or triple-crop based, in which double rice cropping and rice–wheat rotations are the traditional and mainstream patterns. In recent years, the maize–rice cropping system with high yield, high utilization of light and temperature resources, high fertilizer and water efficiency, and low emissions advantages, has been established in this region [1]. Under such intensive cropping systems with large amounts of crop straw produced, straw incorporation into the soil has become a major crop residue disposal [2]. However, the straw-return method is important for specific regional ecological conditions and cropping systems [3], which may result in positive or negative effects on soil fertility and environment [4]. Therefore, great effort should be made to explore a suitable straw-return method and understand its effect on soil quality and crop yields in the maize–rice cropping system.

Many studies have examined the influence of straw incorporation on crop yields [5]. Returning crop straw to soil balances the mineralization-related carbon (C) loss in agricultural soil and improves a range of biological and physiochemical soil properties, thus supporting sustainable crop production [6,7]. Soil total organic carbon (TOC) change rates are twice as high in systems relying upon straw-return (0.29 g kg^{-1} $year^{-1}$) compared with those using chemical fertilizers alone (0.14 g kg^{-1} $year^{-1}$) in Chinese paddy soil [8]. Soil microbial mass (SMB) and bioactivity can be promoted through residue retention efforts. Lou et al. [9] reported that in Northeast China there was significantly higher microbial biomass C under straw retention due to C and nitrogen (N) content improvements as well as soil moisture and porosity increases than under straw removed. Soil microorganisms play critical roles in soil organic matter (OM) decomposition and soil nutrient biogeochemical cycling in the agroecosystem [10]. In addition, the alteration in microbial community structure regulates C and N transformation [11]. Residue retention can release aliphatic acids and humic compounds to disrupt superphosphate (P) adsorption by blocking aluminum oxide adsorption sites, and then increase topsoil P levels [4]. Within plant cells, K^+ is present in a readily usable form, and over 90%–95% of this potassium is released from the residual crop straw over a 90-day period. These results suggest that straw incorporation has the potential to improve soil quality [12] and then to promote crop growth and yield. However, for the conventional straw-return method, crushed straw was incorporated into a 15 cm depth of the soil via rotary tillage, which could negatively affected the seed germination and early growth of crops [13,14]. It was demonstrated that this method of straw return plays an important role in improving soil fertility under straw incorporation conditions.

Several studies have shown that in double-rice or rice–wheat cropping systems, straw-return influences soil properties and crop yields [15,16]. However, the effects of different straw-return methods on soil biochemical characteristics and crop yields, and which straw-return method is the most suitable in the maize–rice cropping system are not clear. In addition, due to the limitation of annual thermal resources, the fallow period between maize harvest and late rice planting is short, with less than one week to return maize straw to the soil, which may have adverse effects on the late rice yield. In the present study, we have explored the mechanisms of straw-return practices on the soil microbial community and biochemical processing in maize–rice cropping systems in the middle reaches of the Yangtze River. The objectives of our study were to: (a) examine the effects of different straw-returning methods on soil properties, microbial community, enzyme activities, and crop yields; (b) explore the interaction between soil biochemical characteristics and microorganisms; and (c) determine an appropriate straw-return method which is optimum for the maize–rice cropping system.

2. Materials and Methods

2.1. Experimental Site

The field experiment was conducted from 2016 to 2018 at Qujialing, Hubei province, China (30°52′ N, 112°50′ E). A subtropical monsoon climate prevailed in the area with a mean annual temperature of 16.2 °C and precipitation of 1140 mm. The upland rice rotation dominates the farmland in this region. A wheat–rice rotation was practised by farmers for decades before the experiment.

2.2. Experimental Design and Field Agronomic Management

Spring maize and rice underwent annual rotation from the start of maize sowing in late March 2016 to the final rice harvest in early November 2018; thus, six successive crops were grown in the same experimental plots. Each experimental plot was 62.4 m^2 (9.4 × 6.5 m). Ridges of 0.5 m in width between adjacent plots were built and were covered with the strong black plastic film to prevent permeable lateral flow. A local maize hybrid (Xingken6) was sown in late March at a density of 60,000 plants ha^{-1}, with 60 cm row spacing, and harvested around July 20 each year. The rice seedlings (Evan17) were transplanted around July 25 at a 13 × 25 cm spacing with a 300,000 hills ha^{-1} density and harvested in early November.

Four treatments were implemented: (1) no straw return (CK), (2) maize straw was removed from the plots and rice straw was incorporated into the soil (M_0R_i), (3) both maize and rice straw were incorporated into the field (M_iR_i), and (4) maize straw was mulched and rice straw was incorporated into the field (M_mR_i). All treatments were arranged in a randomized block design with three replicates. In the CK treatment plots, the above ground portions of straws were removed after the harvest. In the M_0R_i treatment, the maize straw was removed, and the rice straw was chopped into 5–10 cm pieces and added to the 0–20 cm layer via a rotary cultivator. In the M_iR_i plots, the aboveground straw of both maize and late rice was chopped and added to the 0–20 cm soil layer via small rotary cultivation after harvesting. In the M_mR_i plots, after the maize and rice straw were chopped (5–10 cm pieces), maize straw was mulched on the soil surface evenly and the rice was incorporated by rotary tillage to the 0–20 cm soil. The maize and rice straw dry weights were measured via moisture content in oven-dried subsamples.

All treatments were fertilized in the same way (300, 90, and 135 kg ha^{-1} of N, P_2O_5, and K_2O, respectively,) during the maize growing season, consistent with standard local agronomic practices. Fertilizers with 30% N (urea), 50% K (potassium chloride), and 100% P (superphosphate) were applied as a basal fertilizer before maize sowing. The remaining urea was applied 30% at the 6-leaf stage, 40% at 12-leaf stage, and 50% of the remaining potassium chloride were applied at the 12-leaf stage. No irrigation was conducted during the maize seasons. During the rice season, 180 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, and 90 kg K_2O ha^{-1} were applied for each treatment. Thirty percent of the N fertilizer was applied at the seedling stage, 30% at the tillering stage, and 40% at the panicle stage. The 60% K fertilizers (KCl) were applied at the tillering stage, and 40% at the panicle stage. All P fertilizers were used as a basal treatment before transplanting. Other field management, such as for pests, diseases, and weeds, during maize and rice seasons was conducted in accordance with conventional management.

2.3. Sampling and Analysis

At harvest, ears of 50 maize plants were collected from the middle rows in each plot to determine the grain yield. Five replicate areas (5 m^2 per plot) were assessed to determine average rice grain yields, and these maize and rice yields were adjusted based upon 0.14 g H_2O g^{-1} standard moisture. The C and N content in maize and rice was measured by the CHNOS elemental analyzer (Vario MAX, Elementar, Germany).

Each year, 0–20 cm soil layer samples were collected from all plots following the maize and rice harvests. Five cores (5 cm diameter) along diagonal lines were collected and pooled for all plots, and these combined samples were divided into four parts, with ~500 g remaining as a subsample for analyses. A 2 mm mesh filter was used as a sieve for fresh soil samples, which were then split into two, with one being stored at 4 °C for microbial biomass carbon (MBC), dissolved organic carbon (DOC), mineralized nitrogen (N_{min}), and pH assessments, and the other being air-dried to assess available P, exchangeable potassium (K), TOC, and total nitrogen (TN).

Soil bulk density (g cm^{-3}) from the 0–20 cm soil layer was measured via a core method [17]. Soil pH was determined via pH meter (Mettler-Toledo FE28, Shanghai Instruments, China) with a 1:2.5 soil:water ratio (*w/v*) [18].

Soil available P was extracted using 0.5 M $NaHCO_3$ with pH 8.5, and measured using the molybdenum blue method [19]. The exchangeable K was extracted using 1 mol L^{-1} ammonium acetate, and measured with flame photometry (FP640, INASA Instrument, China). A CHNOS elemental analyzer (Vario MAX, Elementar, Germany) was used for the TOC and TN measurements, following the passage of samples through a 150 µm mesh screen. KCl (2 M) was used to extract soil NH_4^+–N and NO_3^-–N, and indophenol blue spectrophotometry was used for measurement. N_{min} content is the sum of NH_4^+–N and NO_3^-–N contents.

Soil DOC (mg kg^{-1}) was determined from fresh soil using ultrapure water with a 1:1.7 ratio of soil to water [20]. Soil MBC (mg kg^{-1}) was measured using the chloroform fumigation extraction method using 0.5 mol L^{-1} K_2SO_4 as the extractant [21]. The extract solutions for DOC and MBC were filtered using a 0.45 μm membrane filter. Then, the filtrates were measured using an automated TOC analyzer (Analytik Jena, Germany). Here, the differences in the C contents in the non-fumigated and the fumigated soil samples were calculated to determine the MBC content with a conversion factor (K_{EC}) of 0.45 [22].

For measuring urease activity [23], 0.2 g of soil was allowed to rest for 24 h at 37 °C with 100 μL toluene, 500 μL urea solution, and 1000 μL citrate buffer following air drying, after which 80 μL sodium phenol and 60 μL sodium hypochlorite solution were added. To assess urease activity, the levels of NH_4^+ released (in micrograms) were determined per day per gram of soil at 578 nm with a spectrophotometer. For invertase activity measurements, soil samples (0.1 g) were dried in the air, and then allowed to rest at 37 °C for 24 h along with 15 μL toluene, 250 μL (pH 5.5) phosphate buffer, and 750 μL 8% sucrose. For cellulase activities, 0.1 g of air-dried soil was mixed with 100 μL toluene and 750 μL acetate solution at pH 5.5 under shaking for 3 h at 37 °C, then water bathed at 90 °C for 15 min. The glucose released by invertase and cellulase were then combined with 3,5-dinitrosalicylic acid, and assessed at 540 nm. Invertase and cellulase activities are expressed as mg glucose per g of soil per day. To determine the phosphatase activity, 0.1 g of air-dried soil was mixed with 400 μL 0.5% disodium phenyl phosphate and 50 μL toluene before a 1-day incubation at 37 °C. Phosphatase-released phenol was assessed at 660 nm following a 30 min reaction with 100 μL boric acid buffer and 20 μL chlorodibrominated benquinone imide.

Soil microbial DNA was extracted from 3g of fresh soil samples (3 × 1 g) using the E.Z.N.A.®soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's instructions. The extracted DNA was detected on a 1% agarose gel, and a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA) was used to determine the DNA concentration and purity. The hypervariable region V3–V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5′–ACTCCTACGGGAGGCAGCAG–3′) and 806R(5′–GGACTACHVGGGTWTCTAAT–3′) by an ABI GeneAmp®9700 PCR thermocycler (ABI, Califonia, USA). The fungal ITS-1 region was amplified using the primer pairs ITS1F (5′–CTTGGTCATTTAGAGGAAGTAA–3′) and ITS2R (5′–GCTGCGTTCTTCATCGATGC–3′). The Quantitative Insights into Microbial Ecology pipeline was used to transform the sequencing data as described by Caporaso et al. [24], which were deposited into the NCBI (National Center for Biotechnology Information) Sequence Read Archive (SRA) database.

2.4. Statistical Analyses

The levels of factors found significant in crop yield, soil physicochemical properties, and enzymatic activities among the four treatments were assessed via Tukey's test and results were presented as a letter display. A two-way ANOVA was conducted using the SPSS 24 (IBM, Armonk, New York, USA). The relationship between the composition of bacterial and fungal communities and soil environmental factors was explored with redundancy analysis (RDA) using the vegan package of R [25].

3. Results

3.1. Soil Property

The main soil characteristics taken from the 0–10 cm and 10–20 cm soil layer before experiment are described in Table 1. Before maize was sowed in 2016, the soil TOC and TN were 16.05 g kg^{-1} and 1.69 g kg^{-1} in the 0–10 cm soil layer, and 11.22 g kg^{-1} and 1.29 g kg^{-1} in the 10–20 cm soil layer.

Table 1. Soil properties in different soil layers at the start of the experiment.

Property	Soil Layer (cm)		Measurement Method
	0–10	10–20	
Soil texture	Anthrosols	Anthrosols	Hydrometer Method
pH	6.91	7.14	By pH meter
Bulk density (g cm^{-3})	1.21	1.32	Core sampler method
TOC (g kg^{-1})	16.05	11.22	By CHNOS elemental analyzer
TN (g kg^{-1})	1.69	1.29	By CHNOS elemental analyzer
Available P (mg kg^{-1})	16.85	10.14	By 0.5 M $NaHCO_3$ extraction
Exchangeable K (mg kg^{-1})	230	172	By 1 M ammonium acetate extraction

Note: TOC–total organic carbon, TN–total nitrogen.

3.2. Air Temperature and Rainfall

Rainfall was not regularly distributed throughout the three years (Figure 1). In 2016, the cumulative precipitation during the maize and rice growing season were 1087.6 and 198.2 mm, respectively. The rainfall was 1045.9 mm throughout the 2017 maize and rice season, and 585.3 mm during the maize and rice season in 2018. In comparison with the experimental years of 2016 and 2018, air temperature during the late rice growing season in 2018 was higher, especially during the grain-filling stage, which benefited the yield formation of late rice in 2018.

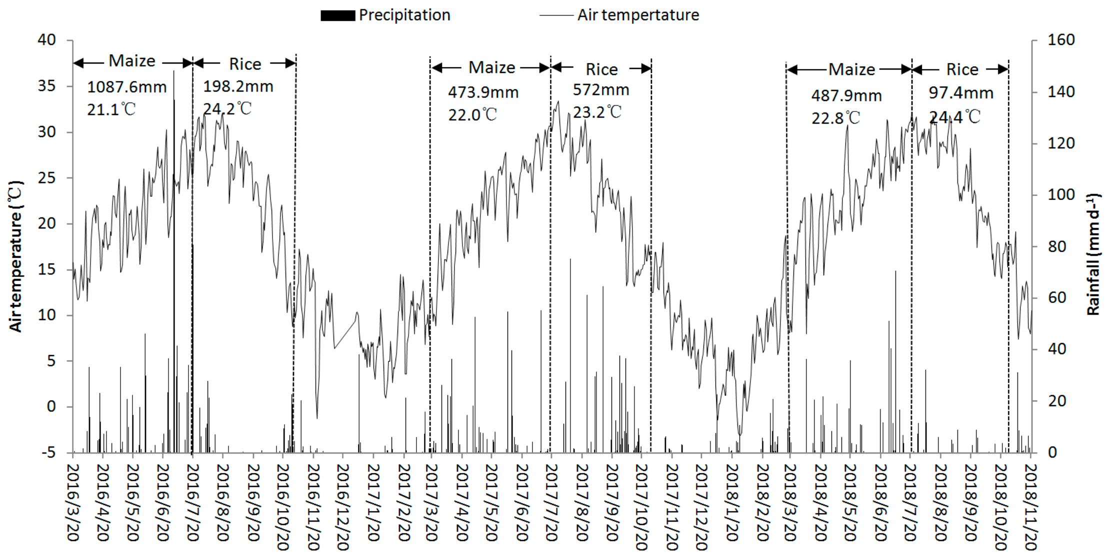

Figure 1. Mean daily air temperature (°C) and rainfall (mm) over the study period from 2016 to 2018.

3.3. Soil pH, BD, TOC and TN

Compared with the 2016 rice harvest season, pH values in CK, M_0R_i and M_mR_i treatments were not significantly different in the 2018 rice harvest season. The soil pH was not significantly different among each treatment in 2016 (Table 2). M_mR_i and M_iR_i treatments brought the soil pH close to neutral in 2017 and 2018, and CK and M_0R_i soil were acidic in 2018. In the 0–10 cm soil layer, bulk density in M_mR_i treatment was significantly decreased in the 2018 rice harvest season, compared with the 2016 rice harvest season (Table 2). In addition, M_mR_i and M_iR_i treatments significantly decreased soil bulk density compared with CK. In the 10–20 cm soil layer, the soil bulk density had no significant difference among four treatments from 2016 to 2018.

In the 0–10 cm soil layer, compared with 2016 rice harvest season, soil TOC under M_0R_i, M_iR_i, and M_mR_i treatments significantly increased by 7.1%, 8.3%, and 8.7% by the 2018 rice harvest season, respectively (Table 2). No significant differences in TOC were detected in CK from 2016 to 2018.

Compared with CK, M_0R_i, M_iR_i, and M_mR_i treatments significantly improved soil TOC by 7.8%, 9.4%, and 10.2% in 2018. We found no significant difference in TOC between M_iR_i and M_mR_i treatments. In the 10–20 cm soil layer, the soil TOC had no significant difference among four treatments from 2016 to 2018.

In the 0–10 cm soil layer, compared with 2016 rice harvest season, soil TN under CK, M_0R_i, and M_iR_i treatments showed no significant difference, while that under M_mR_i treatment significantly increased by 12.2% by the 2018 rice harvest season (Table 2). Compared with CK, M_iR_i and M_mR_i treatments significantly improved soil TN by 3.9% and 4.4% in 2017 and 7.9% and 12.2% in 2018, respectively. In the 10–20 cm soil layer, the soil TN had no significant difference among four treatments from 2016 to 2018.

The interactions among year and straw-return treatment are shown in Table 2. The TOC content in the 0–10 cm soil layer was significantly affected by year, treatment, and interaction. Meanwhile, the TN content in the 0–10 cm soil layer was significantly influenced by year and treatment, while the pH was significantly affected by year.

Table 2. Changes in pH, bulk density, TOC, and TN under different straw incorporation treatments.

	pH	Bulk Density (g cm^{-3})		TOC (g kg^{-1})		TN (g kg^{-1})	
	0–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm
2016 R-H							
CK	6.70 a	1.24 a	1.33 a	15.47 a	11.27 a	1.64 a	1.30 a
M_0R_i	6.73 a	1.23 a	1.36 a	15.52 a	11.36 a	1.70 a	1.31 a
M_iR_i	6.77 a	1.24 a	1.37 a	15.57 a	11.36 a	1.69 a	1.34 a
M_mR_i	6.77 a	1.24 a	1.35 a	15.61 a	11.33 a	1.73 a	1.36 a
2017 R-H							
CK	6.68 c	1.22 a	1.30 a	15.81 a	11.20 a	1.64 bc	1.31 a
M_0R_i	6.76 bc	1.24 a	1.35 a	16.02 a	11.05 a	1.62 c	1.33 a
M_iR_i	7.00 a	1.19 a	1.34 a	16.27 a	10.87 a	1.70 ab	1.32 a
M_mR_i	6.90 ab	1.22 a	1.36 a	15.94 a	11.32 a	1.71 a	1.33 a
2018 R-H							
CK	6.53 b	1.24 a	1.34 a	15.41 b	11.32 a	1.70 b	1.29 a
M_0R_i	6.51 b	1.22 ab	1.37 a	16.62 a	11.42 a	1.69 b	1.33 a
M_iR_i	6.81 a	1.17 b	1.41 a	16.86 a	11.32 a	1.83 ab	1.35 a
M_mR_i	6.70 a	1.19 b	1.40 a	16.98 a	11.97 a	1.90 a	1.36 a
Source of variation							
Year (Y)	*	ns	ns	**	ns	**	ns
Treatment (T)	ns	ns	ns	**	ns	**	ns
Y×T	ns	ns	ns	**	ns	ns	ns

Note: Values were means (n = 3). Different lowercase letters in the same column showed the significant differences between treatments in the same year ($p < 0.05$). R-H, rice harvest. * Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$; ns, non-significant.

3.4. Soil Microbial Community, and the Redundancy Analysis of Soil Microbial Community with Nutrients

The dominant phyla across four treatments were Proteobacteria, Chloroflexl, Actinobacteria, Acidobacteria, Gemmatimonadetes, Bacteroidetes, Firmicutes, and Planctomycetes, accounting for more than 88% of the bacterial sequences from each treatment soil sample (Figures 2 and 3). Ascomycota, Motierellomycota, Rozellomycota, and Basidiomycota phyla were the main fungal phyla in the four treatments, accounting for more than 84%. Unclassified fungal phyla occupied more than 9%. Ascomycota, Motierellomycota, Rozellomycota, and Basidiomycota phyla were significantly influenced by straw-return treatments. Moreover, Ascomycota and Basidiomycota in M_mR_i were observed significantly higher than CK, M_0R_i, and M_iR_i treatments.

The redundancy analysis (RDA) results showed that the relative abundances of Proteobacteria, Actinobacteria, Gemmatimonadetes, Bacteroidetes, Firmicutes, and Planctomycetes could be associated with greater soil properties, such as soil TN, pH, N_{min}, and BD (Figure 3a). The relative abundances of

Ascomycota, Basidiomycota, and unclassified fungal phyla were increased with M_mR_i treatment, which were significantly correlated with the soil TN, MBC, available P, DOC, pH, N_{min}, and exchangeable K (Figure 3b).

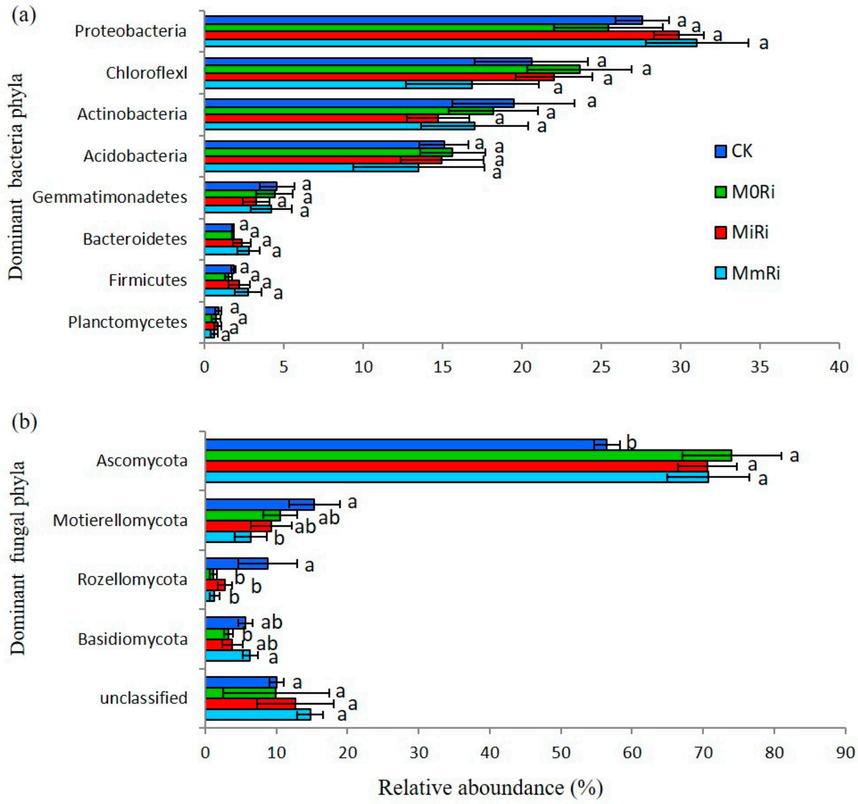

Figure 2. Relative abundance ($n = 3$) of the dominant bacterial and fungal phyla in different straw-return treatments. Relative abundances are based on the proportional frequencies of the DNA sequences that could be classified at the phylum level. We selected the top eight abundant bacteria and top five abundant fungal phyla to show in the bar chart. (**a**) Relative abundance of the dominant bacteria phyla in different straw-return treatments. (**b**) Relative abundance of the fungal phyla in different straw-return treatments. Error bars indicate the standard deviation of the mean.

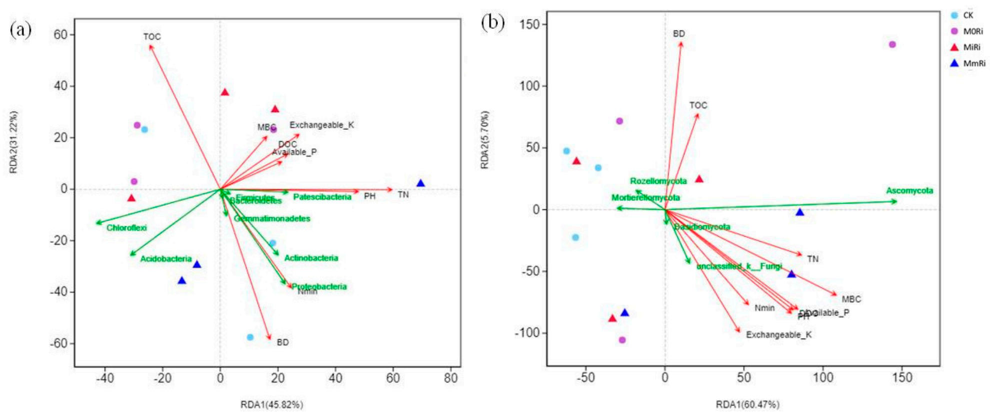

Figure 3. Redundancy analysis of (**a**) the abundant bacterial phyla and (**b**) the abundant fungal phyla orders and nine soil properties (pH, BD, TOC, TN, DOC, MBC, N_{min}, available P, and exchangeable K) of different straw-return treatments. Note: BD, bulk density; TOC, total organic carbon; TN, total nitrogen; DOC, dissolved organic carbon; MBC, microbial biomass carbon; N_{min}, mineralized nitrogen.

3.5. Soil Enzyme Activity

Straw application significantly influenced the soil enzyme activities at the time of the two crops' harvest in 2017 (Figure 4). The M_mR_i treatment had the highest soil enzyme activities. Compared with CK, the M_mR_i treatment remarkably increased soil urease activity by 33.5%, cellulase activity by 37.2%, invertase activity by 12.6%, and phosphatase activity by 8.9% (Figure 4), averaged across two sampling points in 2017. Although soil enzyme activities under M_iR_i treatment were lower that under M_mR_i treatment, compared with CK, the M_iR_i treatment significantly enhanced soil urease activity by 22.0%, cellulase activity by 21.9%, and invertase activity by 6.8% averaged across two sampling points in 2017. Soil phosphatase activity showed a significant difference between M_iR_i treatment and CK at maize harvest; however, no difference was found at rice harvest in 2017. Compared with CK, the M_0R_i treatment exhibited an apparent increase in soil urease and cellulase activities, but no significant changes were detected in soil invertase and phosphatase activities.

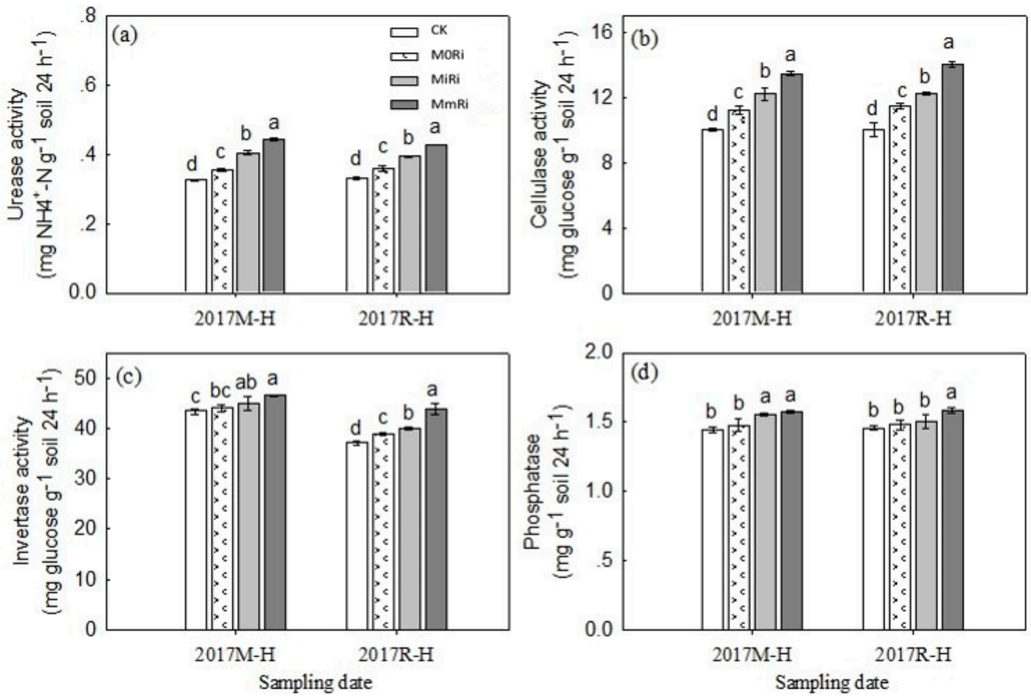

Figure 4. Changes in (**a**) soil urease, (**b**) soil cellulase, (**c**) soil invertase, and (**d**) soil phosphatase activities in the 0–20 cm soil layer under different treatments in 2017. M-H, maize harvest, R-H, rice harvest. Bars with different lower case letters indicate significant differences at $p < 0.05$.

3.6. DOC, MBC, N_{min}, Available P, and Exchangeable K

Active soil organic carbon fractions showed considerable changes under different treatments over time. Straw application obviously increased DOC content immediately after the first year of treatment implementation (Table 3). Compared with the 2016 rice harvest season, CK, M_0R_i, M_iR_i, and M_mR_i treatments significantly increased soil DOC content by 23.7%, 44.7%, 37.0%, and 35.8% in the 2018 rice harvest season, respectively. Compared with CK, the M_0R_i treatment significantly increased DOC content by 18.4%–26.5% at sampling points during 2017 to 2018, whereas M_iR_i and M_mR_i treatments significantly increased it by 42.6%–57.9% and 53.8%–68.9%, respectively. M_mR_i had a higher DOC level than the M_iR_i treatment at all sampling points, with average increase of 7.3%. A similar trend was found in soil MBC (Table 3). Compared with the 2016 rice harvest season, CK, M_0R_i, M_iR_i, and M_mR_i treatments significantly increased soil MBC content in the 2018 rice harvest season. Compared with CK, the M_0R_i treatment significantly increased MBC content by 14.5% to 22.1% in 2017 and 2018, whereas M_iR_i and M_mR_i treatments significantly increased it by 23.3%–33.9% and 30.0%–43.6%, respectively.

All straw-return treatments had considerable effects on soil DOC and MBC in the initial year, which successively amplified in the following years.

Straw application apparently increased soil mineralized nitrogen (N_{min}) content over time (Table 3). Compared with the 2016 rice harvest season, CK, M_0R_i, M_iR_i, and M_mR_i treatments significantly increased the soil N_{min} content by 124.1%, 138.8%, 146.2%, and 167.3% in the 2018 rice harvest season, respectively. Compared with CK, the M_iR_i treatment significantly increased soil N_{min} content at maize harvest, but did not produce apparent increases at late rice harvest in 2017 and 2018. M_mR_i treatment successively showed superiority in soil N_{min} content at the sampling points compared with CK in 2017 and 2018. M_mR_i had higher soil N_{min} levels than M_iR_i treatment at both crop harvests in 2018.

The soil available P content increased gradually over the years (Table 3). Straw-return treatments exhibited significantly higher content of soil-available P than CK since the first year. Compared with the 2016 rice harvest season, available P under CK, M_0R_i, M_iR_i, and M_mR_i treatments increased with the season, and significantly increased by 21.2%, 41.7%, 38.4%, and 41.9% in the 2018 rice harvest season, respectively. At rice harvest in 2018, the M_0R_i treatment increased soil available P by 19.6% relative to CK. Compared with CK, the M_iR_i and M_mR_i treatments significantly increased soil available P content by 17.3%–33.9% and 24.3%–45.6% over the three experimental years, respectively. An increase in soil-exchangeable K content was also observed with straw incorporation (Table 3). Compared with the 2016 rice harvest season, the soil-exchangeable K content under CK and M_0R_i treatments was not significantly different, whereas those of M_iR_i and M_mR_i treatments significantly increased by 22.2% and 19.2% in 2018 rice harvest season, respectively. Compared with CK, the M_0R_i treatment significantly increased soil-exchangeable K content by 7.3% at rice harvest in 2018. The M_iR_i treatment significantly increased soil-exchangeable K content compared with CK by 8.4%–20.1% in the 2017 and 2018 rice seasons. The M_mR_i treatment significantly increased soil-exchangeable K content by 9.1%–23.1% compared with CK over the three years.

The interactions among year, season, and straw-return treatment are shown in Table 3. In the DOC, MBC, N_{min}, available P, and exchangeable K content, significant effects were noted on year, season, and straw-return treatments, and also in the year and straw-return treatment interactions. In addition, the interaction among year, season, and straw-return treatments significantly influenced soil MBC and N_{min} content.

Table 3. Changes in DOC, MBC, N_{min}, available P, and exchangeable K content in the 0–20 cm soil layer under different straw incorporation treatments.

Year	Season	Treatment	DOC (mg kg^{-1})	MBC (mg kg^{-1})	N_{min} (mg kg^{-1})	Available P (mg kg^{-1})	Exchangeable K (mg kg^{-1})
2016	Rice	CK	203.94 b	174.00 c	8.55 a	12.23 c	152.23 b
		M_0R_i	220.56 b	181.52 c	8.31 a	12.51 c	158.14 ab
		M_iR_i	290.77 a	240.68 b	8.35 a	14.35 b	158.06 ab
		M_mR_i	313.75 a	266.60 a	8.43 a	15.21 a	166.07 a
2017	Maize	CK	225.33 d	206.89 c	18.81c	13.48 d	157.04 c
		M_0R_i	266.71 c	236.87 b	19.11 c	14.78 c	165.72 b
		M_iR_i	317.79 b	254.93 a	19.81 b	16.12 b	169.67 b
		M_mR_i	339.66 a	269.03 a	21.00 a	18.14 a	180.98 a
	Rice	CK	232.57 d	235.67 c	19.99 b	14.38 d	167.88 d
		M_0R_i	271.13 c	262.06 c	20.50 b	15.82 c	176.99 c
		M_iR_i	332.08 b	300.18 b	20.44 b	17.57 b	191.65 b
		M_mR_i	362.20 a	333.98 a	22.23 a	19.83 a	205.65 a
2018	Maize	CK	251.74 d	328.72 d	20.27 d	14.54 d	167.55 b
		M_0R_i	316.24 c	388.69 c	23.99 c	16.54 c	169.07 b
		M_iR_i	386.55 b	453.40 b	28.04 b	18.91 b	192.85 a
		M_mR_i	409.60 a	471.56 a	32.40 a	20.31 a	191.24 a
	Rice	CK	252.26 d	347.80 d	19.17 b	14.82 d	160.82 b
		M_0R_i	319.17 c	424.77 c	19.85 b	17.73 c	172.63 b
		M_iR_i	398.25 b	465.77 b	20.56 b	19.86 b	193.22 a
		M_mR_i	426.24 a	499.40 a	22.53 a	21.58 a	198.00 a

Table 3. *Cont.*

Year	Season	Treatment	DOC (mg kg^{-1})	MBC (mg kg^{-1})	N_{min} (mg kg^{-1})	Available P (mg kg^{-1})	Exchangeable K (mg kg^{-1})
	Source of variation						
	Year (Y)		**	**	**	**	**
	Season (S)		**	**	**	**	**
	Treatment (T)		**	**	**	**	**
	Y × S		ns	**	**	ns	**
	Y × T		**	**	**	**	**
	S × T		*	*	**	*	*
	Y × S × T		ns	**	**	ns	ns

Note: DOC, dissolved organic carbon; MBC, microbial biomass carbon; N_{min}, mineralized nitrogen. Values were means (n = 3). Different lowercase letters in the same column showed the significant differences between treatments in the same season ($p < 0.05$). * Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$; ns, non-significant.

3.7. C Accumulation and N Uptake by Crop

The effect of straw return on the crop C and N uptake are shown in Table 4. Straw return significantly increased crop C accumulation and N uptake. Compared with CK, M_iR_i and M_mR_i treatments significantly increased crop C accumulation in the three experimental years by 6.7%–9.5% and 11.2%–13.2%, and increased crop N uptake by 6.9%–15.9% and 14.3%–25.0%, respectively. M_mR_i treatment significantly increased annual crop C accumulation and N uptake by 3.8% and 7.4% compared with M_iR_i.

Table 4. C accumulation and N uptake of maize and rice crops with different straw incorporation treatments in 2017.

Treatment	C Accumulation (kg ha^{-1})			N Uptake (kg ha^{-1})		
	Maize	Rice	Annual	Maize	Rice	Annual
CK	6121 c	6069 b	12190 d	173 c	170 c	344 c
M_0R_i	6475 b	6152 b	12627 c	183 b	170 c	353 c
M_iR_i	6532 b	6646 a	13179 b	185 b	198 b	383 b
M_mR_i	6930 a	6748 a	13678 a	198 a	213 a	411 a

Note: C, carbon; N, nitrogen. Different letters in the same column indicate significant differences between treatments in the same year ($p < 0.05$). Values are mean (n = 3).

3.8. Crop Yield and Amount of Straw Return

Compared with 2017, CK, M_0R_i, M_iR_i, and M_mR_i treatments significantly increased annual crop yield by 28.9%, 25.8%, 31.9%, and 26.3% in 2018, respectively. M_0R_i treatment significantly increased maize yield by 3.1%–13.0% from 2017 to 2018 and rice yield by 2.5%–5.0% from 2017 to 2018, compared with CK. M_iR_i and M_mR_i treatments significantly increased rice yield by 15.7% and 20.6% in 2018 compared with CK, respectively. The crop yield with M_mR_i treatment compared with M_iR_i treatment significantly increased by 6.0%, 5.0%, and 5.1% in 2017 rice season, in 2018 maize season, and in 2018 rice season, respectively. M_iR_i and M_mR_i treatments significantly increased annual crop yield compared with CK by 13.9% and 7.4% in 2017, and 18.2% and 12.9% in 2018, respectively. The straw returned amounts in each treatment are shown in Table 5. From 2016 to 2018, M_iR_i and M_mR_i treatments had significantly higher straw-return amounts than M_0R_i each year. No significant differences were observed between M_iR_i and M_mR_i treatments in 2016, 2017, and 2018.

Maize yield was significantly affected by year, treatment, and by year-by-treatment interaction. Rice and annual yield were significantly affected by year and treatment separately.

Table 5. Crop yield and amount of straw return for maize and rice crops with different straw incorporation treatments from 2016 to 2018.

Year	Treatment	Crop Yield (Mg ha^{-1})			Straw Return (Mg ha^{-1})		
		Maize	Rice	Annual	Maize	Rice	Annual
2016	CK	7.40 a	6.47 b	13.87 a	-	-	-
	M_0R_i	7.54 a	6.30 b	13.84 a	-	8.38 a	8.38 b
	M_iR_i	7.57 a	7.01 ab	14.58 a	5.27 a	8.99 a	14.26 a
	M_mR_i	7.35 a	7.36 a	14.71 a	5.27 a	8.13 a	13.40 a
2017	CK	7.49 b	6.63bc	14.12 c	-	-	-
	M_0R_i	8.32a	6.37 c	14.70 c	-	5.39 a	5.39 b
	M_iR_i	8.33a	7.09 ab	15.41 b	4.87 a	5.39 a	10.25 a
	M_mR_i	8.62 a	7.64 a	16.26 a	5.14 a	5.84 a	10.98 a
2018	CK	8.67 c	9.52 b	18.20 b	-	-	-
	M_0R_i	9.04 bc	9.44 b	18.49 b	-	5.42 b	5.42 b
	M_iR_i	9.65 a	10.68 a	20.33 a	5.13 a	6.51 a	11.65 a
	M_mR_i	9.30 ab	11.25 a	20.55 a	5.27 a	6.50 a	11.77 a
Source of variation							
Year (Y)		**	**	**	-	-	-
Treatment (T)		**	**	**	-	-	-
Y × T		**	ns	ns	-	-	-

Note: Values were means (n = 3). Different lowercase letters in the same column showed the significant differences between treatments in the same year ($p < 0.05$). * Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$; ns, non-significant.

4. Discussion

4.1. Soil pH, BD, TOC, and TN

Straw incorporation into the soil is an important method to increase soil organic matter and nutrients, and finally enhance crop yields. Bulk density is an important physical indicator of soil compaction and changes with agricultural management [26]. Our results demonstrated that crop-straw returning significantly decreased the 0–10 cm soil bulk density, which is consistent with the results of Wang et al. [26] and Mousavi et al. [27]. In addition, the two-season straw-return method increased soil pH, which is consistent with the results of Zhao et al. [28]. Crop straw return is a key to maintaining and/or increasing soil total organic carbon (TOC), which provides insights into the quality of soil and the sustainability of agriculture [29]. Crop straw return can substantially alter microbial environments and affect soil aggregate formation, SOM sequestration within microaggregates, and soil porosity, thus preventing microbial degradation of TOC and increasing TOC content [30]. Many researchers found that long-term application of straw benefits TOC build-up with increases in the annual straw-return rates, thus improving soil fertility [31,32]. We found that straw application increased TOC content over three years (Table 2) in the 0–10 cm soil layer, particularly after two seasons of crop straw return (M_mR_i and M_iR_i treatments). The treatments with higher TOC content decreased soil bulk density, which is consistent with the findings of Wang et al. [26]. More straw was applied each year and underwent decomposition, thus increasing the TOC level, partially by enhancing C input [33]. A meta-analysis showed that straw C input rate positively correlated with TOC in soil significantly [34]. In this study, TN content (Table 2) in M_mR_i treatment increased significantly in the 0–10 cm soil layer, with an average straw return of 12.1 t ha^{-1} year^{-1} (Table 5) over the three years. Zhang et al. [35] found that four-year maize straw return at 13.5 t ha^{-1} year^{-1} significantly increased soil TN content. Researchers reported that 24-year rice straw return increased TN content by 9.18% [31]. In our study, significant increase in TOC and TN contents in 0–10 cm depth of soil was observed after three years of straw returning, which indicated that TOC and TN were gradually changed because of straw returning.

4.2. Soil Microbial Community

Many studies have reported that straw return could alter the soil microbial distribution, and soil microbes determine nutrient turnover, transformation, and cycling in fields [3,36]. Our study suggests that straw return generated greater fungal diversity and similar bacterial diversity compared with CK. Fu et al. [37] also reported that straw return did not increase bacterial diversity at different soil depths. Proteobacteria, Chloroflexl, Actinobacteria, Acidobacteria, Gemmatimonadetes, Bacteroidetes, and Firmicutes were the most common bacterial phyla. Proteobacteria was recognized as copiotrophic taxa that grow at rapid rates in conditions of the increased C and N availability, and play a significant role in C and N cycling [38,39]. Acidobacteria was identified as an oligotrophic taxon that metabolized malnutrition and stubborn C substrate with a slower growth rate [38]. Proteobacteria and Acidobacteria had a positive relationship with soil bulk density, N_{min}, pH, TN, available P, DOC, exchangeable K, and MBC, and negatively correlated with TOC, which is beneficial to C mineralization. Similar results were reported by Fu et al. [37]. In nutrient deficiency and in extreme environmental conditions, Firmicutes could produce dormant spores to live on [40]. In our study, straw returning significantly changed soil fungal distribution in the soil. The main reason is that the straw provides a large amount of the organic carbon for the fungal community. On the other hand, the decomposition of the straw increased the soil N, P, and K, meanwhile promoting the mineralization of soil nutrients. Dai et al. [41] reported that straw return could increase soil nutrient mineralization and reduce heterotrophic microbial activity, therefore, leading to increased fungal growth. Straw returning significantly increased Ascomycota and Basidiomycota in our study. Zhao et al. [11] found that the relative abundances of Ascomycota and Basidiomycota changed along with the soil physical and chemical properties, such as nutrient content, bulk density, and pH. Ascomycota is very sensitive to labile C substrates and has a lower capacity to degrade recalcitrant C. Ascomycota had significant correlations with labile C and N, such as MBC, DOC, and N_{min}, indicating that Ascomycota are involved in organic matter mineralization. Nevertheless, Basidiomycota could generate a range of enzymes to degrade recalcitrant C and has a certain correlation with soil C and N fractions [42].

4.3. Soil DOC, MBC, N_{min}, Available P, Exchangeable K and Enzyme Activity

Crop straw is a substantial source of C, K, and trace elements required for crop growth, and helps maintain the soil nutrient balance after being returned to the field [6]. Crop straw return is able to alter soil DOC and MBC in the short-term [43], given the turnover time of less than one year and the sensitivity to particular management practices [44]. Straw serves as a source of carbon that facilitates microbial growth and the formation of macroaggregates through interactions between soil and the residues in the context of such activity [45], thus contributing to the accumulation of DOC and MBC. Our results demonstrated that both maize and rice straw-return treatments (M_iR_i and M_mR_i) significantly increased soil DOC and MBC (Table 3) in the 0–20 cm soil layer. This may be linked to the release of carbon and other organic compounds from the straw, thereby stimulating local microbial activity [46]. Therefore, the enzymes selected in the current study, i.e., urease, cellulase, invertase, and phosphatase, were significantly activated by straw application treatment in the 0–20 cm soil layer (Figure 4). This result is consistent with Wei et al. [12], wherein urease, invertase, and phosphatase activity in the 0–60 cm soil layer increased with straw addition over five years. Cellulase and invertase significantly increased under straw-return treatments. These enzymes catalyzed the conversion of straw carbon to active organic carbon and increased soil respiration. Our results showed that the maize straw mulch application (M_mR_i treatment) markedly increased the DOC and MBC contents compared with the straw incorporation of the M_mR_i treatment (Table 3). Organic and aliphatic-aromatic acids can be reduced as a consequence of the anaerobic breakdown of residue, leading to toxicity that can impair the growth of rice roots, which might contribute to fewer root exudates. The maize straw surface mulch-return treatment (M_mR_i), compared with straw incorporation treatment (M_iR_i), reduced many negative impacts of this anaerobic activity on the growth of crops. However, further studies are needed to obtain insight into the differences between M_mR_i and M_iR_i treatments in active soil organic C fractions and soil enzyme activities. Soil N_{min} can be used as a standard metric for assessing the fertility of soil [47]. We found that after two years the soil N_{min} content increased considerably under the straw application treatments compared with the no-straw-return treatment (CK), especially under the M_iR_i and M_mR_i treatments with two seasons of straw return (Table 3). This indicates that straw application positively influenced N_{min} in the soil, thereby improving the soil fertility. This is consistent with the findings reported by Wang et al. [48]. Recently-released N from incorporated straw could act as a N resource contributing to N_{min} increase. When maize straw was applied to the soil, the microbes present in this layer were able to act as a sink to immobilize nitrogen compounds by breaking down crop straw and then speeding up the N mineralization [49]. This indicated that with straw decomposition, a portion of straw N could be transformed as N_{min} in soil N cycling [50]. Our findings showed that straw application significantly increased the activity levels of soil urease in soil (Figure 4a). Urease in soil is related to the soil nitrogen cycle, and can catalyze the hydrolysis of urea, hydroxycarbamide, and semi-carbazide to ammonium ions, and promote the mineralization of nitrogen [51]. Additionally, urease transformed the soil N into a source of N available for crop use at a later period, which possibly improves N efficiency [52].

Our findings suggest that straw application significantly increased the available P and exchangeable K contents in soil (Table 3), likely due to crop residue applications as suggested in previous studies [53]. Straw application led to a marked increase in P availability in the top 20 cm of the soil layer, partially due to straw P released into the soil. Our results also showed that straw return significantly increased phosphatase in soil (Figure 4d), which can strengthen the hydrolysis of esters and anhydride of phosphoric acid to release phosphate that plants can directly use [54,55]. In the experiment, straw application appeared to effectively increase the exchangeable K in soil, which was consistent with the findings reported by Sui et al. [56]. Singh et al. [57] also found that residue retention increased soil K content and partially met crop K demands in a rice–maize cropping system. Consistent with Zhao et al. [58], we found that soil pH increased under M_iR_i and M_mR_i treatments (Table 2), which indicated increases in soil K^+ fixation and reductions in K^+ loss by leaching. Increased pH generates new charges in the surface of the soil constant potential and increases the amount of K^+ adsorption, so K^+ more easily replaces Ca^{2+} instead of replacing H^+ and Al^{3+} [59,60]. Straw return may help effectively regulate soil pH within an appropriate range for balancing soil nutrient fixation and recharge [29].

4.4. Crop C Accumulation, N Uptake, Yield and Amount of Straw Return

Straw returning significantly increased crops C accumulation and N uptake, therefore increasing crops biomass and yields. Maize–rice rotation is an intensive cropping system that produces a large amount of straw. About 12.1 Mg straw per hectare was produced under the treatments (M_iR_i and M_mR_i) that two seasons of straw returned each year in our study (Table 5). After three years continuous straw returned, the annual crop yield increased from 11.7% in M_iR_i to 12.9% in M_mR_i compared with CK treatment in 2018, indicating the M_iR_i treatment was the most suitable management practice. Similarly, Liu et al. [34] reported through a meta-analysis that straw return could increase crop yield by an average of 12.3%. Many studies have reported that straw return decreased soil bulk density, buffered surface soil temperatures, and increased soil nutrient, microbial, and enzyme activities, finally providing a favorable chemical, physical, and biological soil environment to benefit the growth of maize and rice [61,62]. In our study, two seasons' straw-return treatments, M_iR_i and M_mR_i changed soil properties and increased crops nutrients, biomass, and yields. However, no increase in crop yield was observed under single rice straw-return treatment (M_0R_i) compared with CK (Table 5). Singh et al. [63] found clear links between straw application and crop yield in seven studies after analyzing 51 rice cropping system datasets. Straw return thus impacts crop yield dependent on soil characteristics, application method, the rate of straw return, and time period. Many previous researchers have reported beneficial outcomes to crop yields from applying crop straw, primarily due to improving the soil physical structure, nutrient content, and microbial activities [54,64].

5. Conclusions

Exploring an appropriate straw-return method for the maize–rice cropping system is essential for increasing crop production in the middle Yangtze River of China. A three-year straw return program improved the soil physical structure and its chemical and biochemical properties significantly, which mainly contributed to overall soil quality improvement and increased crop yields in a maize–rice rotation system. The maize straw mulch return is recommended as a practical measure due to its better soil characteristics and convenient field application that saves time and resources.

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