

Effects of crop rotation and straw return on soil microbiome in a southern paddy field

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ABSTRACT

Crop rotation and straw return are important sustainable agricultural practices. However, their combined impact on the soil microbiota is poorly understood. Here, the effects of crop rotation and straw return on rice (*Oryza sativa* L.) productivity, soil properties and soil microbial community was investigated in a Chinese southern paddy soil. There were six treatments, including rice-fallow (RF), rice-rape (*Brassica campestris* L.) rotation (RR), rice-edamame (*Glycine max* (L.) Merr.) rotation (RE), RF with rice straw return (RFS), RR with rice and rape straw return (RRS), and RE with rice and edamame straw return (RES). The results showed that rice yields in RR and RE were increased by 6.28% and 6.93% relative to RF and straw return group increased by 3.03%-8.16% relative to the group without straw return. Rotation increased soil available P (AP) and RRS significantly increased soil organic matter (OM), total N (TN) and available K (AK) in comparison with RR. Rotation impacts soil microbial community in a stronger way than straw return. Rotation increased the bacterial species numbers and fungal Shannon index. Disease-suppressing bacteria were enriched and some fungal pathogen was decreased in rotation treatments. Straw return had no potential risk in increasing the microbial pathogens. Soil fertilities like AK and AP were closely correlated with bacterial alpha-diversity and rice yield. In conclusion, our research demonstrated that combined crop rotation and straw return is an optimum agricultural practice in increasing rice productivity and soil fertility. The impacts of crop rotation and straw return on the microbiota were distinct. These findings help us manage the crops and residues in southern China.

Key words: *Brassica campestris*, crop rotation, *Glycine max*, microbial community, *Oryza sativa*, paddy soil, straw return.

INTRODUCTION

According to National Bureau of Statistics in Chinese, paddy soil area approximately covered 29.45 million ha which occupies about 25% of the Chinese agricultural field of food crops. China achieved about 0.87 billion tons of crop straw annually (Zhang et al., 2021). Many researchers have proved that straw return can improve soil organic matter (SOM) and nutrient supply resulting in sustainable crop yields (Latifmanesh et al., 2020; Liu et al., 2021). Therefore, straw return has been considered as eco-friendly practice to achieve sustainable agriculture (Dhaliwal et al., 2020).

Soil microorganisms are responsible for the soil stability (Gul et al., 2015) and they are sensitive to soil environmental changes. Crop straw is a valuable C source for microbes. Many researchers have reported the effects of straw on the soil microbiota. Miura et al. (2016) stated that straw return could stimulate the growth of soil microorganisms. The application of straw distinctly influenced the bacterial community under a rice-wheat system (Guo et al., 2016). Su et al. (2020) suggested that corn straw return had negative impact on soil fungal community diversity.

Except for straw return, crop rotation is another common agricultural management practice. Crop rotation is beneficial to overcome continuous cropping obstacle which is common in monoculture (Larkin et al., 2021). Rotation is conducive to improve soil structure (Benitez et al., 2017), increase soil microbial diversity (Woo et al., 2022) and control plant disease (Qin et al., 2022). Paddy-upland rotation changed the environments of soil microbiota and microbial diversity is commonly increased compared to monoculture (Borrell et al., 2017).

However, many studies mainly focused on the effect of a particular management practice on rice productivity, soil fertility and soil microbiome, while ignoring the combined effects of crop rotation and straw return on microbiota. Single farming practice such as crop rotation (Wang et al., 2023) and straw return (Yang et al., 2022) can have obvious impacts on soil microbiota; however, similar responses may be changed when these practices co-occur in an integrated system. Therefore, understanding the impacts of different agricultural management practices on the rice productivity, soil fertility and microbial community will provide valuable information which can facilitate the development of scientific farm management patterns for rice.

The objectives of this study were aimed to (i) investigate the effects of crop rotation and straw return on the rice productivity and soil properties; (ii) elucidate the responses of soil microbial composition and diversities to different management practices; (iii) establish relationships between rice yield, soil fertility, and microbial diversities.

MATERIALS AND METHODS

Site description and experimental design

The study site is located at the Agroecological Experimental Station (26°13' N, 119°04' E) in Minhou County, Fujian Province, southern China. It is located in the transition zone between southern subtropical and central subtropics with a mean annual temperature of 19.5 °C and rainfall of 1350.9 mm. The soil is a yellow clayey soil derived from diluvium. The initial soil had 1.15 g cm⁻³ bulk density, pH 5.28, 20.66 g kg⁻¹ soil organic matter (SOM), 0.97 g kg⁻¹ total N (TN) in 0-20 cm soil layer.

There were six treatments: (1) Rice (*Oryza sativa* L.)-fallow without rotation and straw return (RF), (2) rice-rape (*Brassica campestris* L.) rotation with no straw return (RR), (3) rice-edamame (*Glycine max* (L.) Merr.) rotation with no straw return (RE), (4) RF with rice straw return (RFS), (5) RR with straw return (including rice and rape straw) (RRS), and (6) RE with straw return (including rice and edamame straw) (RES). The experiment field was designed by a split-plot in triplicate per treatment and every plot size was 12 m² (4 × 3 m). Therefore, there were 18 plots in this experiment. The N, P, and K fertilizers were urea, calcium superphosphate and potassium chloride, respectively. The annual amounts of chemical fertilizer are shown in Table 1. All the straws were cut by 5-10 cm and applied to soil at 0-10 cm layer. Rice straw was returned to the field in November, rape and edamame straws were returned in June. The amounts of returned straw, moisture and nutrients of all crop straw were shown in Table 2.

Table 1. Agricultural management practices under different treatments.

Crop	NPK application (kg ha ⁻¹)	Fertilization method	Planting time	Harvest time
Rice	135:54:94.5	N fertilizer: 50% as basal fertilizer, 50% as tillering fertilizer; P fertilizer: 100% as basal fertilizer; K fertilizer: 100% as tillering fertilizer	Late June	Late October
Rape	120:45:112.5	N, P, K fertilizers: 50% as basal fertilizer, 25% as seedling fertilizer, 25% as shooting fertilizer	Early December	Early June
Edamame	90:72:108	N, P, K fertilizers: 50% as basal fertilizer, 50% applied at the podding stage	Late March	Early June

Table 2. The nutrients and moisture contents of crop straw. NDa: No detected, air-dried straw of rice and rape were returned.

Crop	Amount of straw				Moisture content
	return	N	P	K	
	kg ha ⁻¹	%	%	%	%
Rice	3750	0.49	0.09	2.82	NDa
Rape	2381	0.54	0.09	2.79	NDa
Edamame	1319	0.54	0.09	2.85	92.04

Soil sampling and analysis

Soil samples were obtained in October 2019. In each replicate plot, five cores of 0-20 cm were taken and homogenized, resulting in 18 samples (6 treatments × 3 replicates). The soil samples were divided into two parts: One was stored at -80 °C for microbial analysis, and another sample was stored at room temperature for soil analysis. Soil pH was determined by a pH meter (LE438, Mettler-Toledo Instruments, Shanghai, China) in a soil solution (1:2.5 soil:water). The SOM was measured by K₂Cr₂O₇ oxidation method, TN by Kjeldahl digestion, available N with the NaOH hydrolysable method. The available P was extracted using 0.5 mol L⁻¹ NaHCO₃ (pH 8.5) and measured by UV spectrophotometry (UV- 2800A, UNICO, Shanghai, China), available K was extracted using 1.0 mol L⁻¹ ammonium acetate (NH₄OAc, pH 7.0) and determined by flame photometry (Cole-Parmer 2655-00 digital flame analyzer, Chicago, Illinois, USA). All soil properties analysis were followed by the method in Lu (2000).

DNA extraction and sequencing

Soil DNA was extracted with the PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, California, USA). The concentration of DNA was determined by a Nanodrop 2000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, Delaware, USA). The bacterial V3/V4 regions of 16S rRNA was amplified with the primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and fungal ITS1 region was amplified by the specific primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3) and ITS2 (5'-GCTGCGTTCCTTCATCGATGC-3). High-throughput sequencing was implemented on an Illumina MiSeq PE300 platform (Illumina, San Diego, California, USA) at Majorbio Bio-Pharm Technology (Shanghai, China). The raw sequences were submitted in National Center for Biotechnology Information (NCBI, Bethesda, Maryland, USA) Sequence Read Archive database (accession nr: PRJNA848429, PRJNA922620).

Bioinformatic analyses

Raw reads were filtered with QIIME2 (Quantitative Insights Into Microbial Ecology; <https://qiime2.org>) pipeline. Sequences (≥ 97% similarity) were clustered into one operational taxonomic unit (OTU) with UPARSE (Edgar, 2010). The OTUs of the bacteria and fungi were classified through the Silva 138 database (<https://www.arb-silva.de/documentation/release-138.1/>) and Unite 9.0 taxonomic database (<https://unite.ut.ee/>), respectively. Alpha diversity indexes were calculated in Mothur (version 1.30.1). We used R package (R Foundation for Statistical Computing, Vienna, Austria; <https://www.R-project.org/>) "stats" for the comparisons of two groups to screen for differential microorganisms. The functional structures of bacterial and fungal communities were obtained from PICRUST1 and FUNGuild.

Statistical analysis

One-way and two-way ANOVA of soil characteristics and microbial diversities were performed with SPSS (SPSS Statistics for Windows, V26.0, IBM, Armonk, New York, USA) by Duncan's test. Pearson's correlation coefficients of rice yield, soil factors and microbial diversities were also analyzed by SPSS. The non-metric multidimensional scaling (NMDS) and PERMANOVA based on the Bray-Curtis distances were performed to analyze the impacts of crop rotation and straw return on bacterial and fungal communities using the "vegan" package in R.

RESULTS

Rice yield and soil physicochemical properties

In order to avoid the effect of annual climate and management variations on the rice yields, the average rice yields from 2016 to 2019 were calculated. As shown in Table 3, RF treatment had the lowest rice yield with 7692.88 kg ha⁻¹, and crop rotation significantly increased the rice yields ($P < 0.05$). The RR and RE treatments had 8175.84 and 8226.10 kg ha⁻¹ rice yields, respectively. However, no obvious difference in the rice yield between RR and RE was observed. Notably, rice yields in the straw return were obviously increased in comparison with the corresponding treatments without straw return with the range from 3.03% to 8.16%. Two-way ANOVA demonstrated that crop rotation ($P < 0.01$) or straw return ($P < 0.001$) alone had strong impacts on the rice yields, but the combined impact of crop rotation and straw return was not observed.

Table 3. Soil properties and rice yield in different rotation systems with/without straw return. The soils were collected in 2019. SOM: Soil organic matter; TN: total N; AN: available N; AP: available P; AK: available K; RF: rice-fallow; RR: rice-rape rotation; RE: rice-edamame rotation; RFS: RF with straw return; RRS: RR with straw return; RES: RE with straw return. Values are means (\pm SD). Different lowercases in a column indicate significant differences among treatments ($P < 0.05$). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, ^{ns}nonsignificant.

Treatment	SOM	TN	AN	AP	AK	pH	Yield
	g kg ⁻¹	g kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹		kg ha ⁻¹
RF	24.20 \pm 1.13 ^b	1.06 \pm 0.08 ^b	93.5 \pm 13.58 ^b	10.5 \pm 0.98 ^b	28.9 \pm 1.9 ^b	5.09 \pm 0.05 ^a	7692.88 \pm 279.36 ^d
RR	24.07 \pm 1.25 ^b	1.05 \pm 0.09 ^b	104.6 \pm 9.11 ^{ab}	14.4 \pm 3.27 ^a	34.4 \pm 9.9 ^b	5.02 \pm 0.13 ^a	8175.84 \pm 98.86 ^c
RE	23.34 \pm 1.15 ^b	1.02 \pm 0.08 ^b	89.6 \pm 11.12 ^b	14.1 \pm 1.20 ^{ab}	30.0 \pm 6.9 ^b	5.04 \pm 0.08 ^a	8226.10 \pm 135.25 ^{bc}
RFS	25.62 \pm 0.38 ^b	1.13 \pm 0.03 ^{ab}	97.1 \pm 3.66 ^b	12.0 \pm 1.12 ^{ab}	38.8 \pm 1.9 ^b	5.04 \pm 0.09 ^a	8320.29 \pm 102.57 ^{abc}
RRS	28.35 \pm 1.44 ^a	1.28 \pm 0.13 ^a	121.5 \pm 11.67 ^a	13.8 \pm 1.66 ^{ab}	71.7 \pm 14.9 ^a	5.01 \pm 0.16 ^a	8514.72 \pm 52.81 ^a
RES	24.35 \pm 2.01 ^b	1.06 \pm 0.13 ^b	93.2 \pm 11.83 ^b	14.0 \pm 1.75 ^{ab}	37.7 \pm 9.9 ^b	5.07 \pm 0.07 ^a	8475.49 \pm 59.91 ^{ab}
Two-way ANOVA							
Rotation (R)	*	^{ns}	**	*	**	^{ns}	**
Straw (S)	**	*	^{ns}	^{ns}	**	^{ns}	***
R x S	^{ns}	^{ns}	^{ns}	^{ns}	*	^{ns}	^{ns}

The soil properties in 2019 are shown in Table 3. Compared to RF, RR treatment increased the contents of soil AN and AP. After straw return, only rice-rape rotation (RR treatment) improved SOM, TN, AN, and AK in comparison with the treatment without straw return demonstrating that rice-rape system was better than rice-edamame system in improving the soil fertilities. It was worth noting that soil AK content in RRS treatment was almost two times than those of other treatments. However, the pH variations among all treatments were not observed.

Microbial community diversity affected by crop rotation and straw return

After filtering raw data with low quality, a total of 865 402 and 1 269 351 valid 16S rRNA and ITS sequences were achieved and the average lengths were 414 and 233 bp, respectively. The sequence numbers of 16S rRNA and ITS were 40 898-55 263, and 56 573 to 86 603 for each sample, respectively. The OTU numbers of bacteria and fungi were 5475 and 2063, respectively.

The sequences coverage of 18 samples reached over 97.14%, suggesting that the sequence depth is sufficient to meet the analysis requirement. As shown in Table 4, RR treatment observed the highest Chao1 (3879) and ACE indices (3852), suggesting that rice-rape rotation could increase the bacterial community richness relative to RF (Table 4). Two-way ANOVA showed that crop rotation rather than straw return had significant impact on the Chao1 and ACE indices ($P < 0.05$) of bacteria (Table 4).

Table 4. Soil bacterial alpha diversity indices. RF: Rice-fallow; RR: rice-rape rotation; RE: rice-edamame rotation; RFS: RF with straw return; RRS: RR with straw return; RES: RE with straw return. * $P < 0.05$; ^{ns}nonsignificant.

Treatment	Chao 1	ACE	Shannon	Coverage
RF	3675 ± 53 ^b	3708 ± 46 ^b	6.55 ± 0.03 ^a	0.9727 ± 0.0003 ^a
RR	3879 ± 140 ^{ab}	3852 ± 98 ^{ab}	6.65 ± 0.13 ^a	0.9715 ± 0.0009 ^a
RE	3754 ± 47 ^{ab}	3801 ± 26 ^{ab}	6.62 ± 0.01 ^a	0.9724 ± 0.0005 ^a
RFS	3710 ± 109 ^{ab}	3743 ± 78 ^{ab}	6.53 ± 0.15 ^a	0.9723 ± 0.0006 ^a
RRS	3909 ± 156 ^a	3885 ± 131 ^a	6.65 ± 0.04 ^a	0.9711 ± 0.0014 ^a
RES	3841 ± 51 ^{ab}	3837 ± 64 ^{ab}	6.62 ± 0.05 ^a	0.9714 ± 0.0009 ^a
Two-way ANOVA				
Rotation (R)	*	*	ns	
Straw return (S)	ns	ns	ns	
R × S	ns	ns	ns	

The responses of fungal richness and diversity were significantly different from those of bacteria. Rotation practice had no distinct influence on the fungal Chao1 and ACE indices compared with RF. After straw return, RRS treatment was beneficial to increase the fungal species in comparison with RR. Fungal Shannon indices were generally increased in the rotation treatments relative to RF by 22.46%, and 26.67%, respectively (Table 5). After straw return, RES treatment decreased the Shannon index in comparison with RE by 20.22%. Two-way ANOVA demonstrated that rotation practice alone exerted distinct impacts on the fungal Shannon index ($P < 0.05$).

Table 5. Soil fungal alpha diversity indices. RF: Rice-fallow; RR: rice-rape rotation; RE: rice-edamame rotation; RFS: RF with straw return; RRS: RR with straw return; RES: RE with straw return. * $P < 0.05$; ^{ns}nonsignificant.

Treatment	Chao1	ACE	Shannon	Coverage
RF	603 ± 6 ^{ab}	600 ± 12 ^{ab}	2.85 ± 0.13 ^{ab}	0.9983 ± 0.0003 ^a
RR	544 ± 69 ^b	521 ± 82 ^b	3.49 ± 0.12 ^a	0.9983 ± 0.0003 ^a
RE	559 ± 28 ^{ab}	550 ± 28 ^{ab}	3.61 ± 0.25 ^a	0.9986 ± 0.0002 ^a
RFS	556 ± 36 ^{ab}	556 ± 30 ^{ab}	2.70 ± 0.10 ^b	0.9983 ± 0.0004 ^a
RRS	664 ± 14 ^a	656 ± 19 ^a	3.60 ± 0.17 ^a	0.9981 ± 0.0009 ^a
RES	584 ± 65 ^{ab}	582 ± 70 ^{ab}	2.88 ± 0.62 ^{ab}	0.9978 ± 0.0009 ^a
Two-way ANOVA				
Rotation (R)	ns	ns	*	
Straw return (S)	ns	ns	ns	
R × S	ns	ns	ns	

Microbial community affected by crop rotation and straw return

The predominant bacterial phyla were largely consistent but little variations in the relative abundances were observed. The dominant phyla (> 1%) include Proteobacteria, Chloroflexi, Actinobacteriota, Acidobacteriota, Firmicutes, Planctomycetota, Desulfobacterota, Myxococcota, Bacteroidota, Nitrospirota, Verrucomicrobiota and Gemmatimonadota across the different treatments (Figure 1A). Firmicutes and Gemmatimonadota were enriched in RE treatment relative to RF, but Nitrospirota abundance was significantly decreased ($P < 0.01$) (Figure 2A). After straw return, RFS treatment significantly decreased Nitrospirota abundance relative to RF (Figure 2B). However, no differential bacterial taxa were observed between RF and RR, RR and RRS, RE and RES treatments. The bacterial dominant genera were also similar across all soil samples (Figure 3A). Differential bacterial genera between RF and rotation practices were also observed, e.g., *Reyranella* and *Desulfatiglans* were decreased, but *Occallatibacter* and *Actinoallomurus* were enriched in RR treatment. *Bacillus*, *Paenibacillus*, *Alicyclobacillus*, *Microbispora*, *Nitrospira*, and *Ammoniphilus* were significantly enriched in RE treatment (Figures 4A, 4B). After straw return, some bacterial genera were significantly decreased in the treatments with

straw. The abundances of *Phaselicystis*, *Chthonomonas*, *Luteitalea* were significantly decreased in the RF system (Figure 5A). The abundances of *Sporomusa* and *Pelotomaculum* were decreased but *Geobacter* and *Desulfovibrio* were increased in the rice-rape system (Figure 5B). In rice-edamame system, *Conexibacter*, *Delftia*, *Aquicella*, *Desulfosporosinus* and *Sporobacter* were significantly decreased and *Rhizomicrobium* were enriched after straw return (Figure 5C).

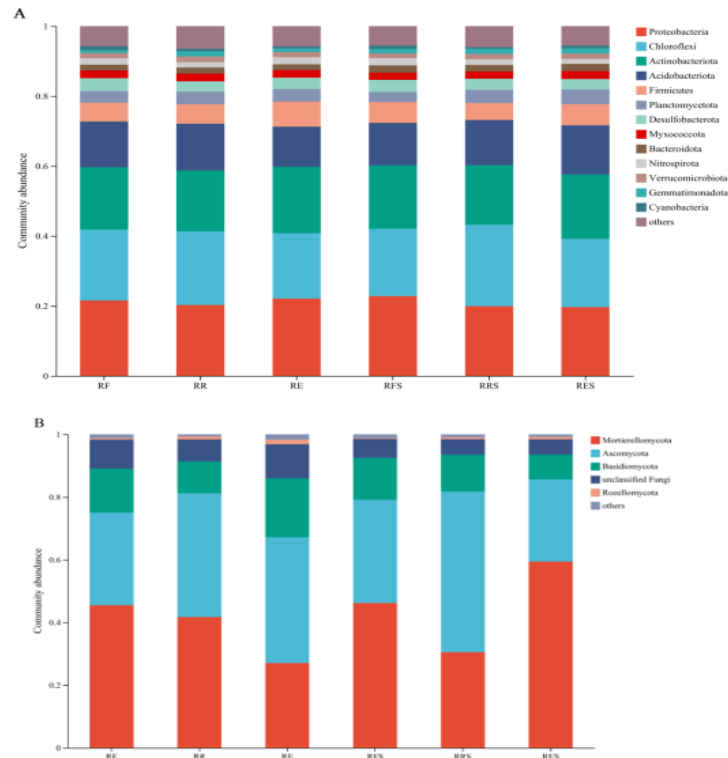


Figure 1. Community composition of soil microorganisms at the phylum level: Bacteria (A), fungi (B). RF: Rice-fallow; RR: rice-rape rotation; RE: rice-edamame rotation; RFS: RF with straw return; RRS: RR with straw return; RES: RE with straw return.

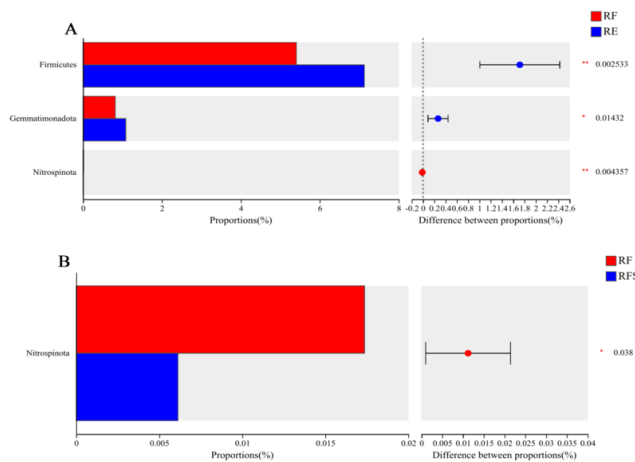


Figure 2. Differential bacterial taxa at the phylum level under different management practices: Rice-fallow (RF)/rice-edamame rotation (RE) (A), RF/RF with rice straw return (RFS) (B). * $P < 0.05$, ** $P < 0.01$.

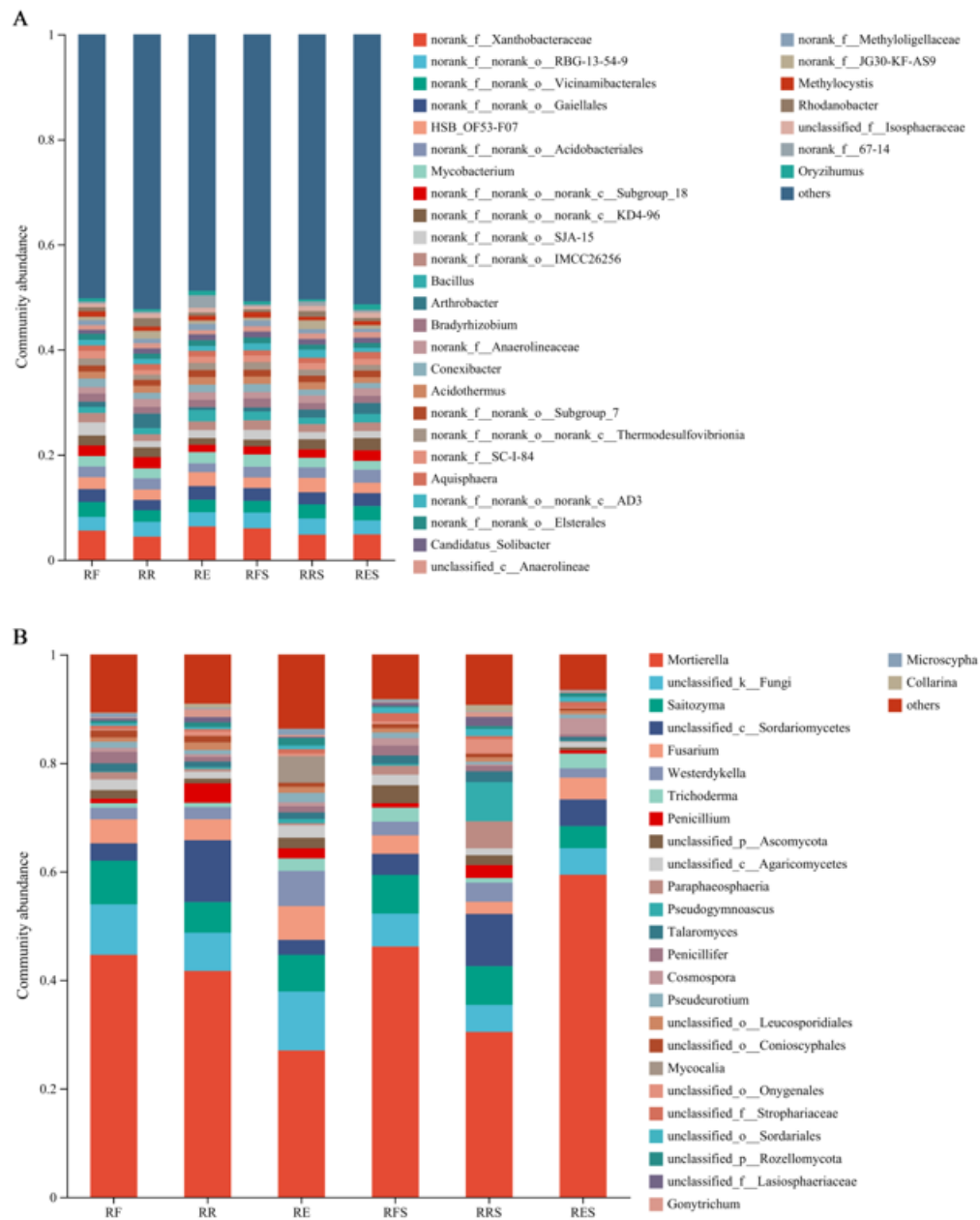


Figure 3. Community composition of soil bacteria (A) and fungi (B) at the genus level. RF: Rice-fallow; RR: rice-rapeseed rotation; RE: rice-edamame rotation; RFS: RF with straw return; RRS: RR with straw return; RES: RE with straw return.

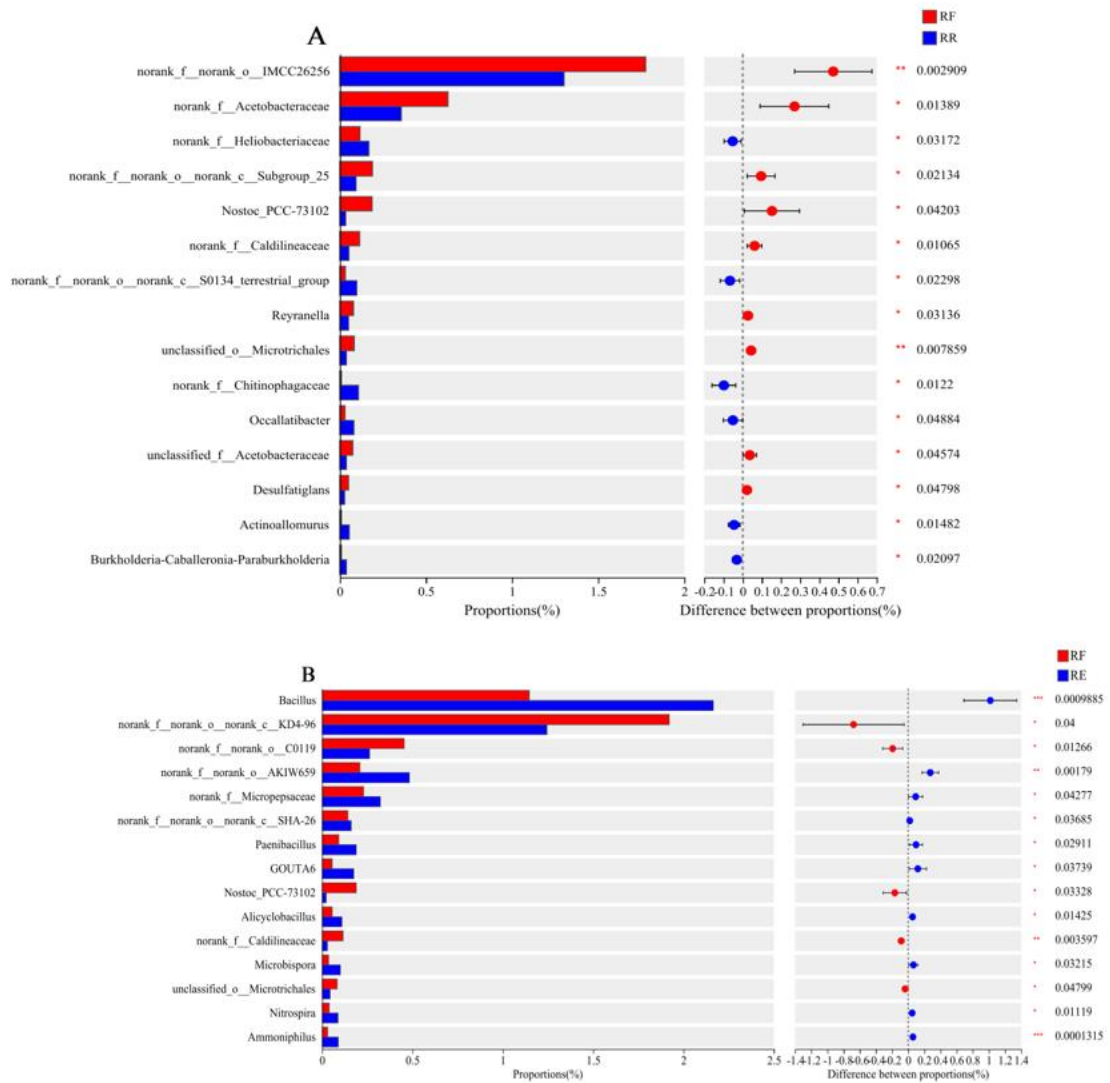


Figure 4. Two groups of bacterial comparison based at the genus level between rice-fallow (RF) and rotation practices. RF: Rice-fallow; RR: rice-rape rotation; RE: rice-edamame rotation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

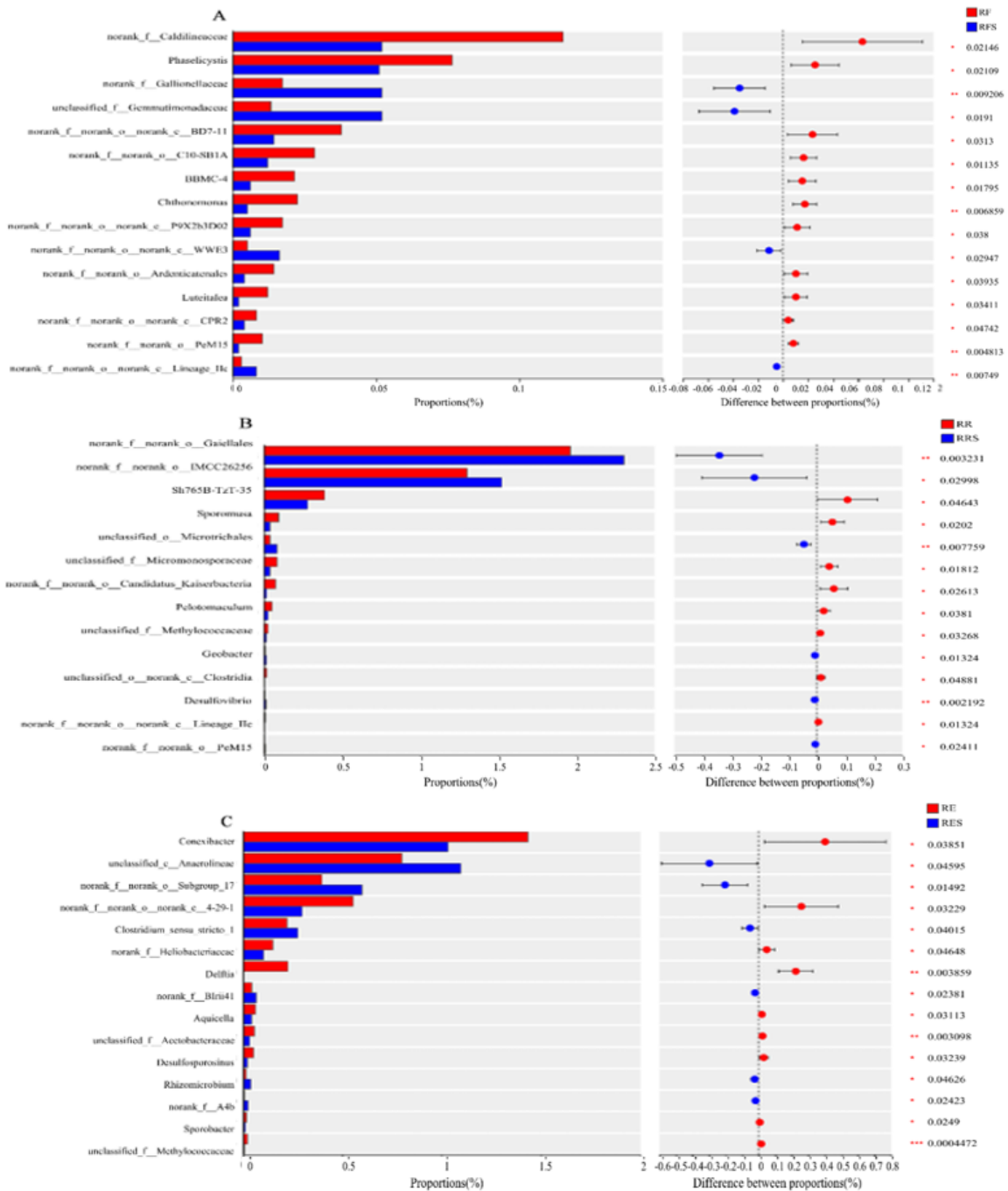


Figure 5. Two groups of bacterial comparison based at the genus level between treatments with and without straw return. RF: Rice-fallow; RR: rice-rape rotation; RE: rice-edamame rotation; RFS: RF with straw return; RRS: RR with straw return; RES: RE with straw return. * $P < 0.05$, ** $P < 0.01$.

For fungal community, the predominant phyla were classified as Mortierellomycota, Ascomycota, Basidiomycota with 26.04% to 59.35%, 26.21% to 51.20% and 7.92% to 18.81% across all samples, respectively (Figure 1B). Crop rotation and straw return resulted in some differential fungal phyla. Compared with RF, RR treatment decreased Basidiomycota and Kickxellomycota abundance at 5% significance level (Figure 6A). The RE treatment decreased Mortierellomycota abundance ($P < 0.05$) but enriched Rozellomycota relative to RF ($P < 0.01$) (Figure 6B). After straw return, RFS significantly increased the abundance of Zoopagomycota relative to RF ($P < 0.01$) (Figure 6C). In comparison with RE, RES treatment decreased Chytridiomycota abundance ($P < 0.05$) (Figure 6D). *Mortierella* was the most abundant genus, and its abundances had big variations across all samples (Figure 3B). The abundance of *Mortierella* was decreased in RR and RE relative to RF. In comparison with RF, RR treatment decreased the abundances of *Paraphaeosphaeria*, *Rhizophyidium* and *Lycoperdon* (Figure 7A). And for RE treatment, the abundances of *Paraphaeosphaeria* and *Collarina* were significantly decreased but *Thielavia*, *Phialocephala*, *Phialosimplex* were increased (Figure 7B).

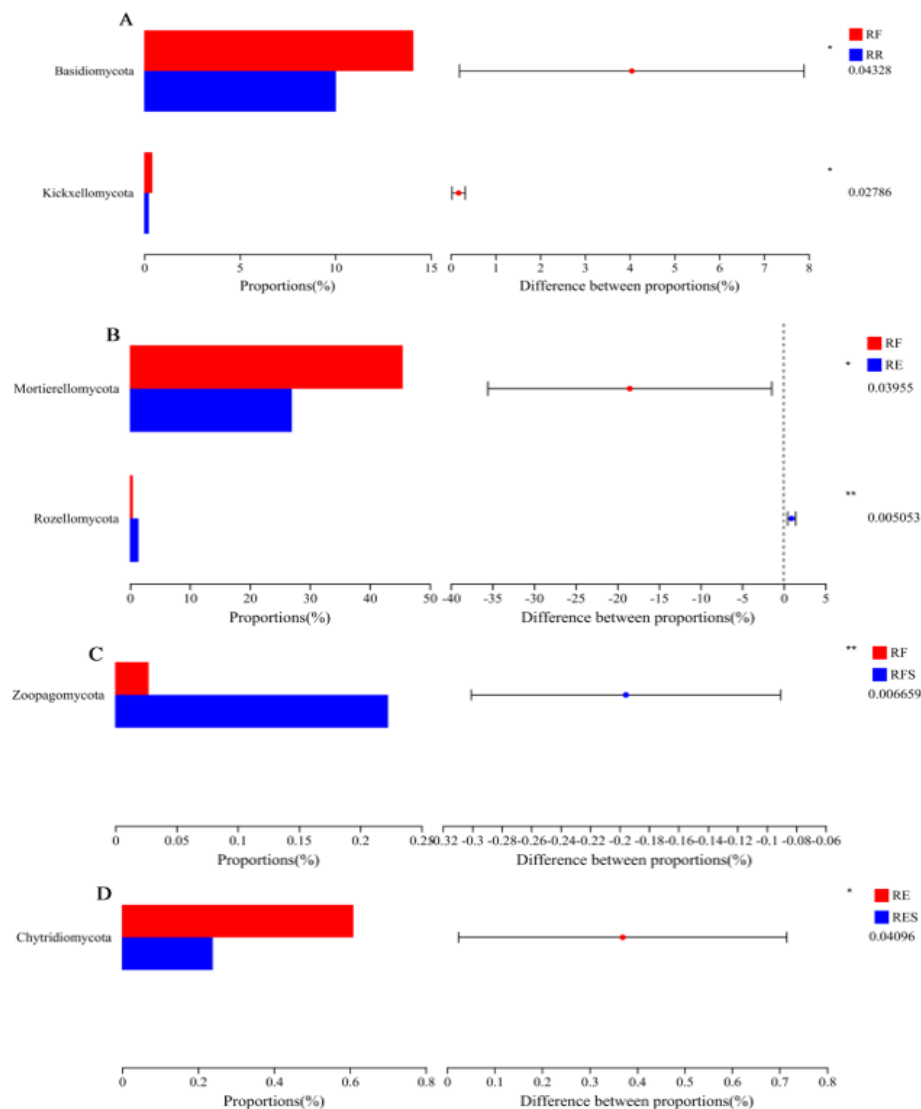


Figure 6. A-B: Differential fungal taxa at the phylum level between rice-fallow (RF) and rotation treatments. C-D: Differential fungal taxa between treatments with and without straw return. RR: Rice-rape rotation; RE: rice-edamame rotation; RFS: RF with rice straw return; RRS: RR with straw return; RES: RE with straw return. * $P < 0.05$, ** $P < 0.01$.

After straw return, the differential fungal taxa between the group with and without straw return were analyzed, e.g. *Trichoderma* was enriched in RFS relative to RF (Figure 8A), and RRS significantly decreased the abundance of fungal pathogen *Fusarium* relative to RR ($P < 0.05$) (Figure 8B). Moreover, genus *Chaetomium* was enriched in RES treatment in comparison with RE (Figure 8C).

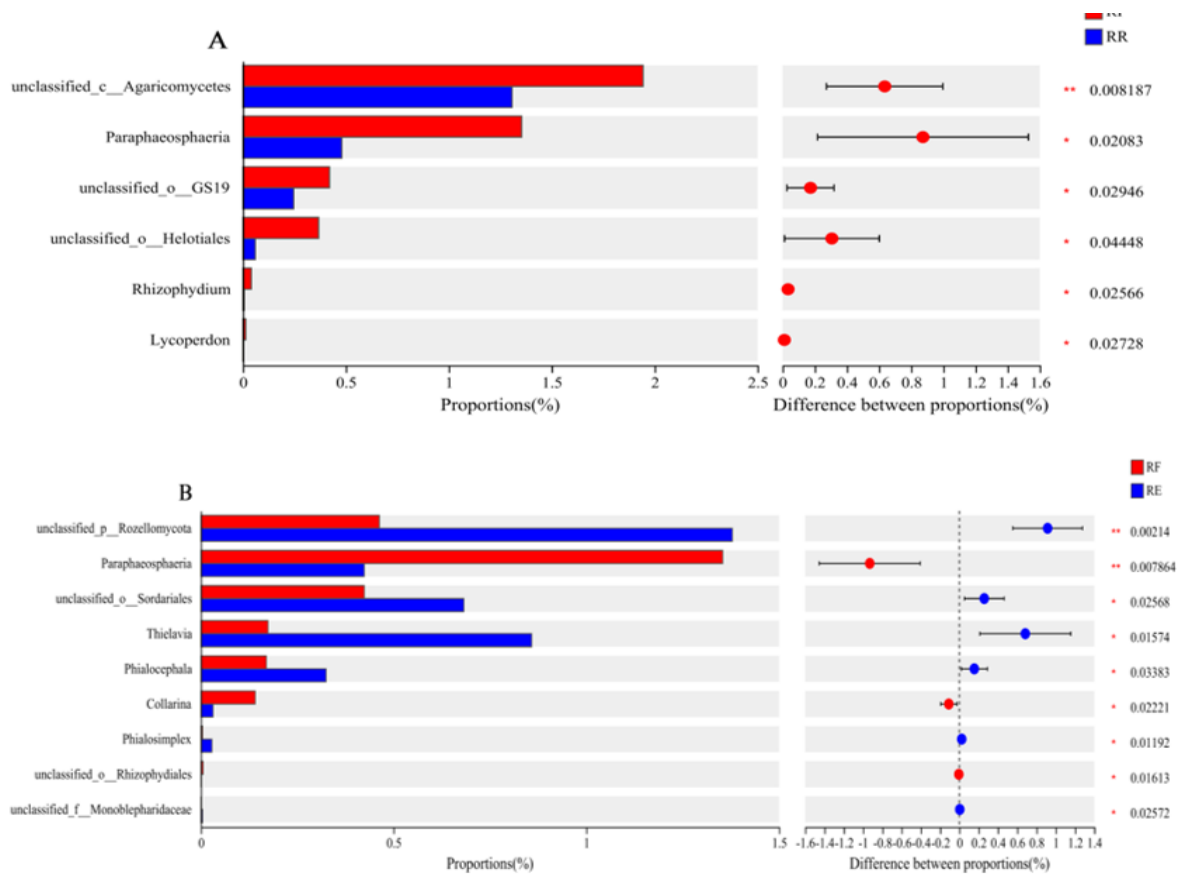


Figure 7. Two groups of fungal comparison based at the genus level between rice-fallow (RF) and rotation practices. RR: rice-rape rotation; RE: rice-edamame rotation. * $P < 0.05$, ** $P < 0.01$.

Non-metric multidimensional scaling (NMDS) of soil colonies

In Figure 9A, subsamples points of RR treatment had the farthest distance from RF and RE, which demonstrated that there was obvious separation in the soil bacterial community between RR and RF, RE. For fungal community, there was no overlap among RF, RR and RE, suggesting that the rotation type had a distinct effect on the fungal community (Figure 9B). Bacterial and fungal communities between the groups with and without straw return in the same rotation system were also evaluated (Figures 10A-10F). The non-metric multidimensional scaling (NMDS) ordinations revealed that the application of straw had no evident impact on the soil microbiota. Above results demonstrated that crop rotation rather than straw return had obvious influence on the soil microbiota. Furthermore, the PERMANOVA test also yielded the same results (Table 6). Crop rotation practice exerted a significant effect on the bacterial ($r^2 = 0.3398$, $P < 0.05$) and fungal ($r^2 = 0.3614$, $P < 0.05$) community structures. However, the dispersion of triplicate samples decreased the influence of crop rotation on the microbiota.

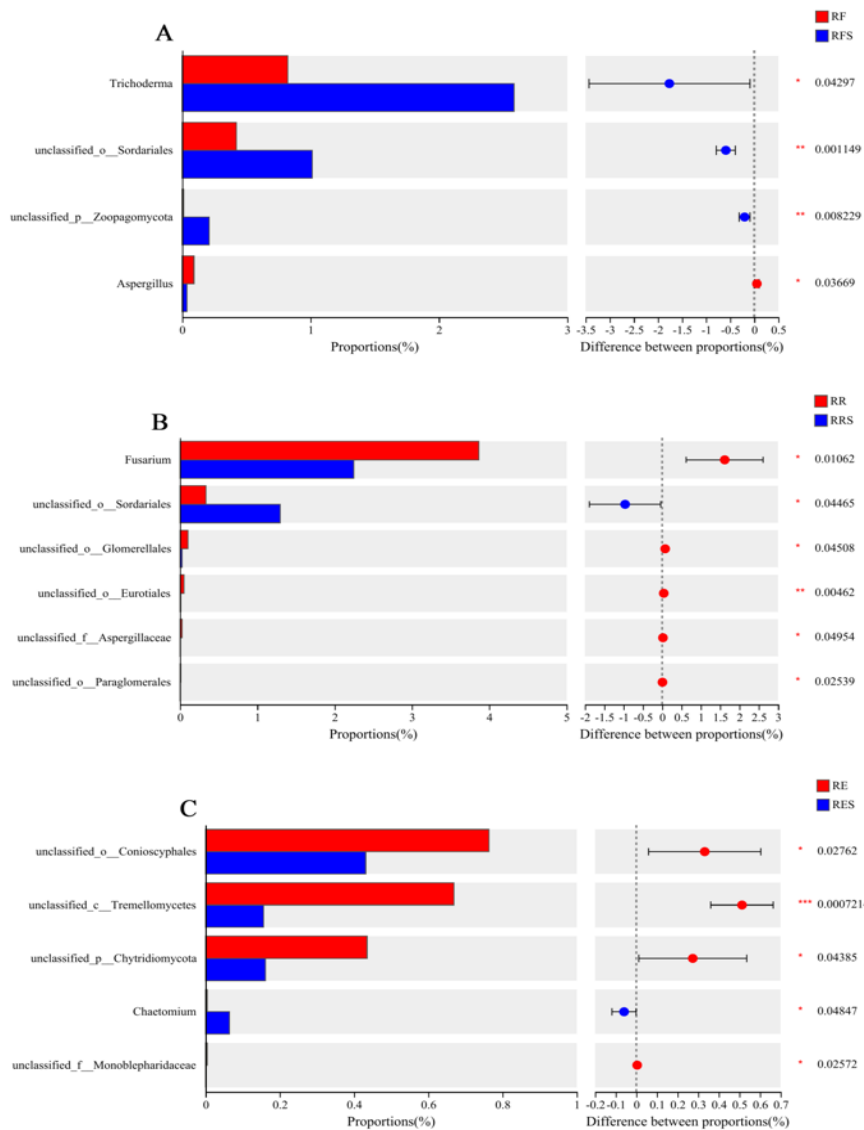


Figure 8. Two groups of fungal comparison based at the genus level between treatments with and without straw return. RF: Rice-fallow; RR: rice-rape rotation; RE: rice-edamame rotation; RFS: RF with straw return; RRS: RR with straw return; RES: RE with straw return. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

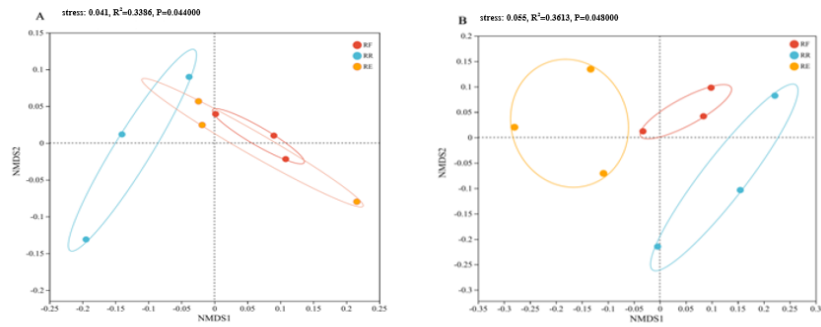


Figure 9. Non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarities were calculated at the operational taxonomic unit (OTU) level: Bacteria (A), fungi (B). RF: Rice-fallow; RR: rice-rape rotation; RE: rice-edamame rotation.

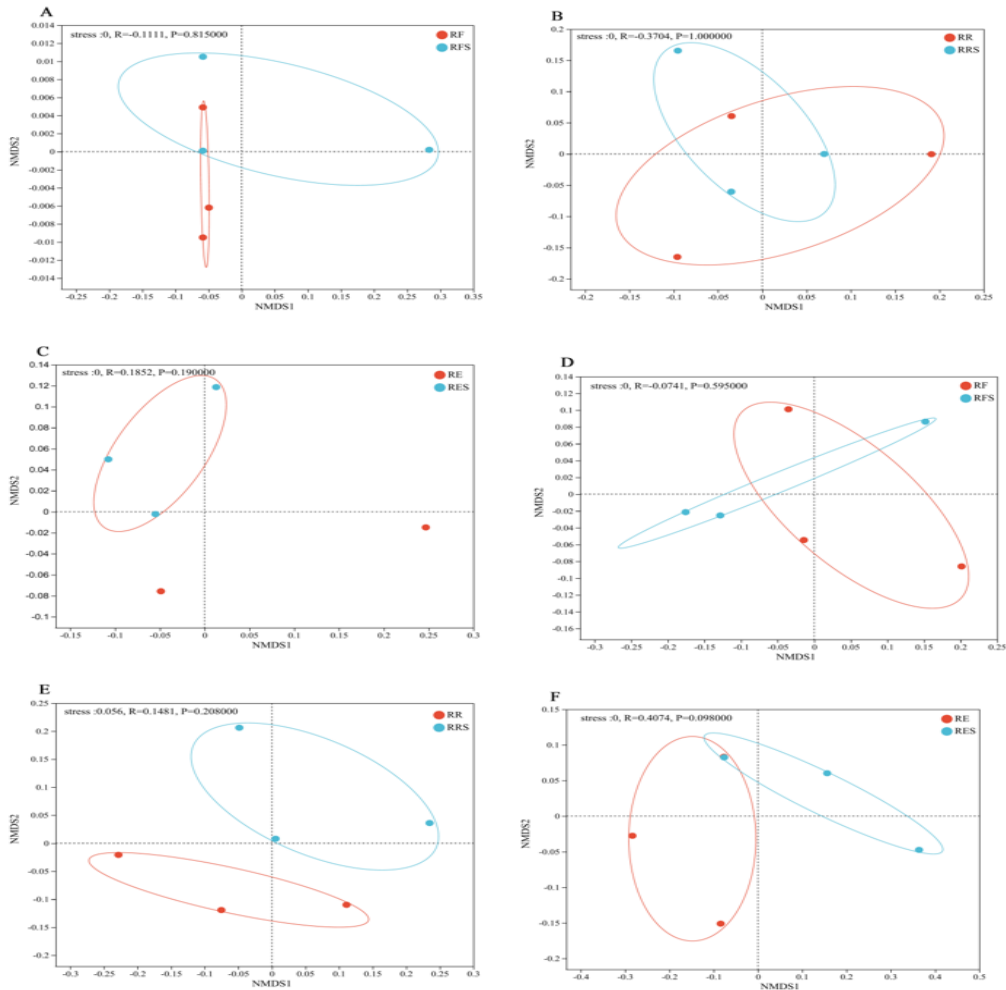


Figure 10. Ordination plots showing the influence of straw return on bacterial (A-C) and fungal (D-F) community structures in the same rotation mode. Non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarities were calculated at the operational taxonomic unit (OTU) level. RF: Rice-fallow; RR: rice-rape rotation; RE: rice-edamame rotation; RFS: RF with straw return; RRS: RR with straw return; RES: RE with straw return.

Table 6. The PERMANOVA for the effects of crop rotation and straw return on soil bacteria and fungi. * $P < 0.05$. RF: Rice-fallow; RFS: RF with rice straw return; RR: rice-rape rotation; RRS: RR with rice and rape straw return; RE: rice-edamame rotation; RES: RE with rice and edamame straw return.

Characteristics	Community	r^2	P -value
Crop rotation	Bacteria	0.3398	0.034*
	Fungi	0.3614	0.045*
RF/RFS	Bacteria	0.1854	0.7
	Fungi	0.1422	0.6
RR/RRS	Bacteria	0.1623	0.9
	Fungi	0.2219	0.2
RE/RES	Bacteria	0.2233	0.4
	Fungi	0.4000	0.1

Microbial community functions affected by crop rotation and straw return

Amino acid transport and metabolism was the abundant function for bacteria, followed by energy production and conversion, and signal transduction mechanisms (Figure 11A). No noticeable alterations in the relative abundances of functions were observed across all soil samples (Figure 11B).

The FUNGuild database showed that endophyte-litter saprotroph-soil saprotroph-undefined saprotroph was the predominant fungal ecological functional (Figure 11C). Crop rotation and straw return both exerted influences on the relative abundances of fungal functional groups. Crop rotation practice decreased the relative abundance of endophyte-litter saprotroph-soil saprotroph-undefined saprotroph. The RE treatment (6.22%) significantly increased the abundance of animal pathogen-endophyte-lichen parasite-plant pathogen-soil saprotroph-wood saprotroph relative to RF (4.42%); however, RR decreased its abundance (3.87%). These results demonstrated that rice rotation with edamame may have the potential to increase the animal and plant pathogen but rotation with rape can be decreased the pathogen. After straw return, the relative abundance of animal pathogen-endophyte-lichen parasite-plant pathogen-soil saprotroph-wood saprotroph was decreased in RFS (3.40%), RRS (2.25%) and RES (4.01%) relative to RF (4.42%), RR (3.87%), and RE (6.22%), respectively. This indicated that straw return did not increase the risk of the transmission of pathogen from crop straw to the paddy field.

Correlations between rice yield, soil properties and microbial diversities

Soil AK content was significantly positively correlated with microbial Chao1, ACE indices and rice yields (Table 7 and 8). Moreover, soil AP was significantly positively correlated with bacterial Chao1, ACE, Shannon indices and rice yields (Table 7). But for fungal community, soil AP was only significantly positively correlated with Shannon index and rice yields (Table 8).

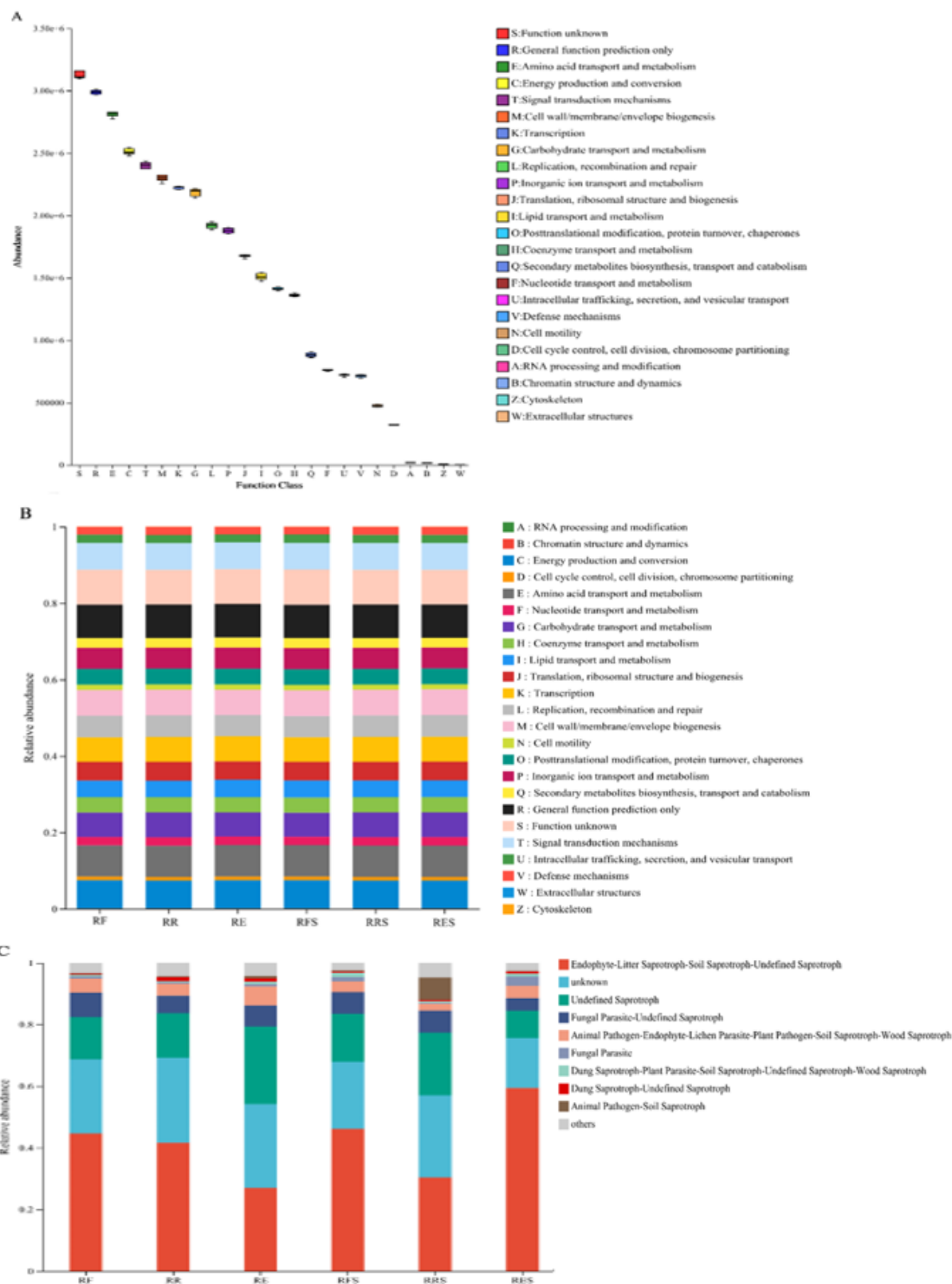


Figure 11. The microbial functional prediction: Clusters of Orthologous Groups (COG) functional classification box diagram (A), functional histogram of soil bacteria (B), fungal functional prediction (C). RF: Rice-fallow; RR: rice-rape rotation; RE: rice-edamame rotation; RFS: RF with straw return; RRS: RR with straw return; RES: RE with straw return.

Table 7. Correlations of soil properties, rice yield and bacterial diversities. * $P < 0.05$, ** $P < 0.01$. OM: Organic matter; TN: total N; AN: available N; AP: available P; AK: available K.

	R^2	Chao1	ACE	Shannon	Yield
OM		0.484*	0.450	0.264	0.436
TN		0.523*	0.511*	0.247	0.425
AN		0.499*	0.517*	0.267	0.405
AP		0.692**	0.639**	0.548*	0.480*
AK		0.646**	0.654**	0.301	0.535*
pH		-0.357	-0.358	0.140	-0.219
Yield		0.489*	0.516*	0.337	

Table 8. Correlations of soil properties, rice yield and fungal diversities. * $P < 0.05$. OM: Organic matter; TN: total N; AN: available N; AP: available P; AK: available K.

	R^2	Chao1	ACE	Shannon	Yield
OM		0.630*	0.650*	0.186	0.436
TN		0.625*	0.638*	0.246	0.425
AN		0.500	0.546	0.531	0.405
AP		-0.267	-0.202	0.586*	0.480*
AK		0.641*	0.665*	0.342	0.535*
pH		-0.384	-0.388	-0.393	-0.219
Yield		0.081	0.079	0.240	

DISCUSSION

Rice yield and soil properties in different treatments

Our 4-yr field experiment indicated that rice rotation with green manure, e.g., rape and edamame could significantly increase the rice yield (Table 3). Growing green manure crops in the fallow season in rice production have been documented as beneficial for improving rice productivity (Zhou et al., 2016). Enhancing biodiversity (like crop rotation) may improve C sequestration and soil fertility (Ditzler et al., 2021). Zheng et al. (2016) presented that paddy-upland rotation can facilitate the soil C cycle and the decomposition of SOM resulting in improved soil fertility. In our study, crop rotation improved soil AP content (Table 3). Furthermore, RR system had the best performance in improving soil available nutrients. Incorporation of the green manure into the soils can fix N_2 , activate soil nutrients to achieve high soil available nutrient (Mbuthia et al., 2015). Previous reports suggest that rape can improve soil fertility, reduce fertilizer application and maintain high rice yield in rice cropping systems (Huang et al., 2017).

Moreover, straw return also increased the rice yield in different rotation system relative to the treatment without straw return by 8.16% (RF), 4.14% (RR), and 3.03% (RE), respectively (Table 3). The remarkable result is the highest rice yield found in the combined rotation crop and straw return, which was verified by Yang et al. (2019) who reported the highest rice yield was observed when returning combined leguminous and gramineous residues to the field. In our study, RRS treatment benefits the improvement of soil OM, TN, AN and AK compared with RR (Table 3). Straw returning contributes to improve the SOC pool and nutrient supply (Liu et al., 2014). The nutrients immobilized by straw would be released across the crop growing season and then improve the nutrients uptake for the crop. Soil AK in RRS treatment was almost two times relative to RR. According to the Chinese second National Soil Census, soil AK content in RRS treatment was belonged to the level four (50-

100 mg kg⁻¹), however, other treatments were belonged to the level five (30-50 mg kg⁻¹). Li et al. (2016) showed that rape straw returning to the paddy field can not only replace partial K, but also improve soil available K.

Compared with the single rotation mode, combined crop rotation and straw return had obvious advantages in improving the rice yield and soil fertility indicating that combined management practice is favorable in southern paddy field and RRS is the optimum planting mode with the highest rice yield and soil fertility.

Microbial diversity in different treatments

Soil organic C sources mainly consisted of root exudates and crop residues impact the soil microbiota. Our results demonstrated that crop rotation changed the soil bacterial and fungal diversity. Notably, crop rotation increased bacterial community diversity (Chao1, ACE) and RR had the most bacterial species (Table 4). The responses of fungal community diversity were apparently different to bacteria, crop rotation increased the fungal Shannon indices (Table 5). But Xi et al. (2021) reported that cotton/maize rotation increased the bacterial diversity and decreased fungal ones.

In our study, there were no apparent variations in bacterial diversity after the application of straw regardless of rotation type (Table 4). For fungal community, RRS increased the fungal species in comparison with RR. Meanwhile, fungal Shannon in RES was lower than that of RE (Table 5). Above results demonstrated that straw returning to the field had a stronger impact on soil fungal diversity than bacteria. In the process of straw decomposition, bacteria predominate in the early stage because they can grow rapidly on the available compounds of the fresh plant residue (Paterson et al., 2008). Fungi can degrade more recalcitrant substances dominating in the later stages (Marschner et al., 2011). In our study, our soil samples were collected several months after straw returning into the field when only recalcitrant substances were present. Therefore, the soil fungi should be more sensitive to straw return than the bacteria.

Microbial communities in different treatments

The top major bacterial phyla were Proteobacteria, Chloroflexi, Actinobacteriota, and Acidobacteriota which were also detected in previous studies (Xia et al., 2019). However, some differences were observed in soil subject to different crop rotation systems, e.g. RE increased Firmicutes and Gemmatimonadota abundances (Figure 2A). Different C sources from the crop will develop the specific microbiota in the rhizosphere soil (Edwards et al., 2015) and it will also impact the microbiota in the bulk soil. Rotation practices increased the relative abundances of *Actinoallomurus*, *Bacillus*, *Paenibacillus*, and *Microbispora* relative to RF (Figure 4A, B). Genera *Bacillus* and *Paenibacillus* have the capability to control plant disease (Oliševska et al., 2019). *Microbispora* was considered as biocontrol tool against fungal pathogens (Das et al., 2021). Pozzi et al. (2011) reported that the strains of *Actinoallomurus* could produce antibiotics suggesting *Actinoallomurus* is a good source of novel antibiotics. These beneficial microbes enriched in the rotation system can improve the disease-suppressing ability of the crop which maybe contributed to improve the rice yield. Meanwhile, genus *Nitrospira* which is involved in the N cycle was enriched in the RE treatment in comparison with RF (Figure 4B).

After straw return, some distinct bacterial genera were observed in different rotation system. The RFS significantly decreased the abundance of pathogen *Phaselicystis* (Figure 5A) suggesting that the practice of straw return in the rice-fallow system is beneficial. Masuda et al. (2017) reported that genus *Geobacter* is the predominant driver of N-fixing in the paddy field. The RRS increased the abundance of *Geobacter* in comparison with RR (Figure 5B). Higher abundance of *Geobacter* may be beneficial to increase the N content in the RRS treatment in comparison with RR (Table 3).

For fungi, Mortierellomycota, Ascomycota and Basidiomycota were the dominant phyla (Figure 1B). Fungal functions are classified as saprotroph, pathotroph and symbiotroph. Saprophytic fungi can convert complex organic substances into simple components, which are closely related to soil C sequestration (Sun et al., 2016). Soil saprotrophic fungal community is mainly composed of Ascomycota and Basidiomycota which play important roles in decompose organic matter. After straw return, RFS and RRS increased the total abundances of Ascomycota and Basidiomycota (Figure 1B). The pathogenic fungi, *Paraphaeosphaeria* was obviously decreased in the rotation group compared to RF (Figures 7A, 7B). Genus *Phialocephala* was obviously increased in RE treatment (Figure 7B). Hu (2019) reported that some species of *Phialocephala* had the capability of dissolving P, and higher AP content in the RE treatment than that of RF was also observed (Table 3).

After straw return, RFS significantly increased the relative abundance of *Trichoderma* (Figure 8A). *Trichoderma* are widely known biostimulants which can boost the crop yield and health (López-Bucio et al., 2015). Meanwhile, RFS could decrease the pathogen *Aspergillus*. In the rice-rape system, RRS significantly decreased the abundance of pathogen *Fusarium* relative to RR (Figure 8B). *Fusarium* spp. are important plant pathogens (Munkvold, 2017). Above results demonstrated that straw return could increase the beneficial fungi but inhibit the detrimental ones. Meanwhile, RES increased the abundance of genus *Chaetomium* (Figure 8C). Members of the genus *Chaetomium* can decompose cellulose and yield diverse bioactive metabolites (Wang et al., 2016). Our results suggest that crop rotation had distinct impact on the soil microbiota and fungi was more sensitive to crop rotation (Figure 9). This result is consistent with the finding by Cassman et al. (2016), who reported that fungal community are closely associated with plant in long-term fertilized grassland. Overall, crop rotation was the main factor in shaping soil microbiota, while straw return with less impact.

CONCLUSIONS

This study determined the responses of soil properties and microbiota to the crop rotation and rice straw return. Combined crop rotation and straw return had huge advantages in increasing the rice yield and soil fertilities in southern China and rice-rape rotation with rice and rape straw return is considered as the best agricultural management practice with the highest rice yield and soil fertilities. The impacts of rotation and straw return on the bacterial and fungal communities were distinct. Crop rotation plays a key role in shaping the soil microbiota. Moreover, fungi were more sensitive to crop rotation than bacteria. Crop rotation increased bacterial Chao 1 and ACE indices and fungal Shannon index. Soil available P and available K were highly related with the microbial diversities and rice yield. Both crop rotation and straw return could increase beneficial microorganisms but decrease the soil pathogens. Incorporation of crop rotation and straw is essential to maintain sustainable agricultural developments.

Author contributions

Conceptualization: F.W. Methodology: Q-H.L., C-M.H. Validation: C-M.H. Formal analysis: Y.F. Investigation: Q-H.L., K.W. Resources: Q-H.L. Writing-original draft: Y.F. Writing-review & editing: K.W. Supervision: F.W. Funding acquisition: F.W. All co-authors reviewed the final version and approved the manuscript before submission.

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