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1 **Paddy-upland rotation regimes shape soil microbial communities and functional traits**

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Abstract

Aims Crop rotation systems profoundly influence soil microbial communities, yet their effects on bacterial and fungal diversity, structure, and function under paddy-upland rotations remain unclear. This study aims to elucidate how winter cover crops alter soil microbial assembly and potential functions, and identify key drivers.

Methods We established six rice-based rotation regimes (rice-wheat, rice-rape, rice-hairy vetch, rice-barley, rice-faba bean, and rice-fallow) in a field experiment. Bacterial and fungal communities were characterized via 16S rRNA and ITS amplicon sequencing, followed by α/β diversity, functional prediction, and correlation analyses with soil properties and yield.

Results Bacterial diversity declined significantly in rice-rape rotation during winter, while fungal diversity was lowest in rice-fallow during the rice season. Rice-legume (hairy vetch, faba bean) and fallow shared similar community structures, whereas rice-rape differed distinctly. Legume rotations enriched carbon and nitrogen cycling bacteria (e.g., Bacteroidota, Planctomycetota) and the predatory Myxococcota, along with organic-degrading genera (*Ellin6067*, *Terracidiphilus*) and the predatory *Haliangium*. Rice-rape significantly enriched the denitrifier *Rhodanobacter*, while rice-rape and rice-legume accumulated pathogenic *Fusarium*. Functional predictions tightly mirrored structural changes. Soil pH, total carbon, and nitrogen dominated bacterial community variation in the rice season, while inorganic nitrogen and bulk density were key in winter. *Rhodanobacter* and *Nitrosospira* correlated positively with inorganic nitrogen. Crop yield was positively associated with carbon-nitrogen-cycling microbes but negatively with pathogens.

Conclusion Rotation regimes drive effects on microbial composition, diversity, and function through modifications of soil physicochemical properties. Our findings provide a scientific basis for designing rotations that balance soil health and stable production.

Keywords

Rice paddies; Agricultural practices; Soil microbial community; Microbial diversity; Function prediction

Introduction

Diversified crop cultivation serves as an effective approach to stabilize agricultural production and enhance sustainable development (Yan et al., 2025). Sustainable agriculture relies on the multifunctionality of soil, particularly the efficient operation of critical processes such as nutrient cycling, organic matter decomposition, and carbon (C) sequestration (Wang et al., 2025). The maintenance of these processes depends on soil bacteria and fungi—the core organisms regulating the C-nitrogen (N) cycle and nutrient supply (Long et al., 2025). However, the understanding of how cropping regimes affect soil microbial communities remains limited, especially regarding their mechanisms in rice-wetland rotation systems where anaerobic and aerobic environments are frequently alternated.

Paddy-upland rotation is one of the main planting regimes of rice in eastern and south Asian countries (Zheng et al., 2016), particularly in the Yangtze River Basin of China (Hao et al., 2025). The traditional double-cropping system with rice and wheat has led to the decline in soil resource utilization efficiency and biodiversity due to the long-term planting of single winter wheat, which limits the yield potential (Banjara et al., 2021; Jat et al., 2019). By contrast, planting green manure crops in winter can

improve soil organic carbon storage, promote N supply and improve soil structure, which are expected to significantly improve the ecological benefits and production potential of the paddy-upland rotation system (Garland et al., 2021; Xie et al., 2016; Zhou et al., 2022). However, research on the effects of winter green manure crops on soil microbial abundance, diversity, and function in the current season and the subsequent rice season is not sufficient, which restricts further optimization and sustainable management of this planting system.

The application of green manure crops through root exudates and biomass return not only provides abundant nutrients for soil microorganisms but also alters their community composition and functional activity (D'Acunto et al., 2024; Yang et al., 2020; Zhou et al., 2020). These microbial changes may simultaneously promote beneficial microorganisms while it may potentially facilitate the spread of pathogenic species (Shao et al., 2024; Sun et al., 2024), though the understanding of their positive and negative effects remains incomplete. Furthermore, the dynamic soil conditions in water-dry rotation systems complicate this process (Li et al., 2024). Therefore, systematic research on how different winter crops influence soil microbial community structure, functions, and ecological effects under water-dry rotation management is crucial for balancing ecological benefits and disease risk mitigation.

Based on the rice-upland double cropping system, this study investigated the microbial characteristics under six winter crop systems: rice-wheat (R-W), rice-rape (R-R), rice-hairy vetch (R-H), rice-barley (R-B), rice-faba bean (R-F), and rice-fallow (R-Fa). Utilizing amplicon sequencing, we analyzed microbial community composition and diversity in soils across these systems. Moreover, we identified key environmental factors driving variations in microbial community, in combination with soil physicochemical property analysis. The findings aim to clarify the residual effects of different winter crops on soil microbiota, providing scientific evidence for optimizing winter crop systems to enhance productivity and ecological stability.

Materials and methods

Site description

The field study was conducted in the Gewu Experimental Station of the Jiangsu Coastal Area Institute of Agricultural Sciences, Yancheng, Jiangsu Province, China (119.97°E, 33.16°N). The climate of this region is subtropical monsoon with an annual mean air temperature of 13.7 °C, annual precipitation of 1051 mm, annual sunshine of 2200 h, and frost-free period of 210 days. The soil in this area is classified as Anthrosols (IUSS Working Group WRB, 2015). The basic characteristics of the topsoil (0–20 cm depth) at the beginning of the experiment were as follows: soil organic carbon (SOC) 11.38 g kg⁻¹, total nitrogen (STN) 1.27 g kg⁻¹, available phosphorus 15.20 mg kg⁻¹, available potassium 63.40 mg kg⁻¹, and pH 7.53. Since 2010, this area has consistently practiced a rice-wheat rotation each year, with straw being returned to the soil.

Experimental design and agronomic measurement

This study was based on a long-term field experiment established in June 2016. The field experiment comprised of six treatments: rice-wheat (*Triticum aestivum* L., R-W), rice-rape (*Brassica campestris* L., R-R), rice-hairy vetch (*Vicia villosa* Roth., R-H), rice-barley (*Hordeum vulgare* L., R-B), rice-faba

bean (*Vicia faba* L., R-F), and rice-fallow (R-Fa). The experimental plots sized 10 m × 4.8 m, were laid out in a randomized complete block design with three repetitions. Rice was direct-seeded in mid-June after rotary tillage to a depth of approximately 15-20 cm. Flood irrigation was applied during most of the rice-growing period, while intermittent drainage was conducted at the tillering and grain-filling stages according to local agronomic practices. After rice harvest, rice straw was evenly retained on the soil surface as mulch under no-tillage conditions. Winter crops were then seeded in treatments R-W, R-R, R-H, R-B, and R-F. The harvest dates were as follows: wheat in early June, rape in late May, barley in early June, and fresh faba bean pods in early May. The aboveground residues of hairy vetch and faba bean were cut and returned to the soil in early May, while wheat, rape, and barley straw were incorporated after grain harvesting. Mineral N, P and K fertilizers were applied in the form of urea (46% N) and a compound fertilizer (15% N, 15% P₂O₅, and 15% K₂O). The application rate of fertilizer and planting density for each crop have been shown in the previous research (Zhang et al., 2022).

Soil sampling, DNA extraction and sequencing

To determine soil microbial diversity and community structure, soil samples from the plow layer (0–20 cm) were collected during the rice harvest period in October 2020 and before the incorporation of green manure in May 2021. The visible impurities were quickly removed and sieved using a 2 mm sieve, then transported to the laboratory in an ice box as soon as possible and stored in a refrigerator at -80 °C for later testing. The structural characteristics of soil microbial communities were determined by the amplicon sequencing method. The subsequent DNA extraction and sequencing were performed by Novogene Co., Ltd. (Beijing, China). The process is as follows: Genomic DNA from the soil sample was extracted by the CTAB method (Zhang et al., 2024a), and the purity and concentration of the DNA were assessed using agarose gel electrophoresis; then the sample was diluted with sterile water to 1 ng µl⁻¹. The amplification of the bacterial 16S rRNA V3-V4 region was performed using specific barcoded primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') (Zhu et al., 2021) and 907R (5'-CCGTC AATTCCTTTGAGTTT-3') (Zhao et al., 2008), while fungi were quantified using the ITS2 primers ITR-H (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Yu et al., 2022). PCR products were detected by electrophoresis using a 2% agarose gel, and then the qualified PCR products were purified by magnetic beads and quantified using an enzyme label. For the target strip, the gel recovery kit (Tiangen Biotech (Beijing) CO., LTD) was used to recover the products. The TruSeq® DNA PCR-Free Sample Preparation Kit was used to build the library. The constructed library was quantified using Qubit and Q-PCR; then it was sequenced on the Illumina NovaSeq platform (NovaSeq6000, Illumina, San Diego, CA, USA).

Statistical analysis

Before the analysis of variance (ANOVA), normality of the data was tested. The data were log-transformed when they were not normally distributed. The ANOVA and LSD were conducted using R software (version 4.5.1) with the “*agricolae*” package. The residual distribution was examined after ANOVA. Differences among treatments were considered statistically significant when $P < 0.05$.

The processing and analysis of soil microbial DNA sequencing data were carried out through the

NovoMagic Platform (<https://magic.novogene.com>), and the main steps are as follows: Each sample's data were separated from the offline data according to the Barcode sequence and PCR amplification primer sequence. After cutting off the barcode and primer sequences, FLASH (V1.2.7, <http://ccb.jhu.edu/software/FLASH/>, Magoč et al., 2011) was used to splice the reads of each sample. The effective tags were then obtained by filtering the splicing sequence and removing the chimeric sequences. The Uparse algorithm (Uparse v7.0.1001, <http://www.drive5.com/uparse/>, Haas et al., 2011) was used to cluster effective tags from all samples, and the sequences were clustered into OTUs (Operational Taxonomic Units) with 97% identity. The OTUs sequences were annotated, and species annotation analysis was conducted using the Mothur method with the SSUrRNA database (Wang et al., 2007) from SILVA138 (<http://www.arb-silva.de/>, Edgar et al, 2013) to obtain taxonomic information (with a threshold of 0.8~1) and generate statistics on the community composition of each sample at each classification level. MetaStat analysis was conducted at each classification level using R software to obtain species with significant differences between treatments. Qiime software (Version 1.9.1) was used to calculate the Alpha (α) indices: Observed OTUs, Chao1, Shannon, Simpson, ACE, Goods coverage, PD_whole_Tree index, while Tukey and Wilcox rank-sum tests were employed to analyze the significance of microbial diversity differences between groups. To assess microbial community complexity and compare variations between samples (or groups), beta (β) diversity analysis was performed using Qiime software. The Unifrac distance was calculated using Qiime software, and the differences between groups for the beta diversity index were analyzed with R software. Adonis analysis (Permutational Multivariate Analysis of Variance, PERMANOVA) was performed using functions in the R “*vegan*” package. Functional prediction analyses of bacterial and fungal communities were performed using Tax4Fun and FunGuild software, respectively, to obtain the relative abundance of functions, and functional differences between treatments were analyzed using T-test. Tax4Fun is a functional prediction based on the metabolic pathway KEGG database using 16S sequencing data, and function is divided into Level 1 (biological process categories) and Level 2 (subdivided functional pathways) on this basis. At the OTU level, the “*vegan*” package in R software was used to conduct a canonical correspondence analysis (CCA) of species abundance and environmental factors. Then, the “*psych*” package and the “*pheatmap*” package in R software were further used to conduct Spearman correlation analysis on the α diversity and dominant populations (genus level) of bacteria and fungi with the tested key soil indicators. Soil bulk density (BD), pH, total carbon (STC), SOC, STN, ammonium N ($\text{NH}_4^+\text{-N}$), nitrate N ($\text{NO}_3^-\text{-N}$), rice yield and annual aboveground biomass were selected as the environmental factors. The testing methods and results of these factors are presented in previous research (Zhang et al., 2022), except for $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ during the winter season, which are shown in Table S1.

Results

Microbial diversity

The α diversity of soil bacteria and fungi under different cropping systems is shown in Table 1. Specifically, the results showed that the number of bacterial species in the rice and wheat seasons is 3301~3470 and 3326~3675, respectively, while the number of fungal species is 352~488 and 513~582, respectively. Additionally, the Goods_coverage of bacteria and fungi reached more than 98%, indicating that almost all species in the sample were covered. Regarding inter-group differences,

analysis showed no difference in bacterial α -diversity among the treatments in the rice season. In contrast, some significant differences were found in the wheat season ($P < 0.05$). For example, the observed_species of bacteria were significantly lower in R-R than in R-Fa treatment, and both Shannon and Simpson indices were significantly lower in R-R than in other treatments during the wheat season. Furthermore, the PD_whole_tree index under R-Fa treatment was significantly higher compared to that in R-R and R-B treatments.

As for fungi, the observed_species, ACE, and PD_whole_tree under R-Fa treatment had the lowest value and were even significantly lower compared to those under R-B treatment in the rice season ($P < 0.05$). The Shannon and Simpson index under the R-F treatment was lower than that under other treatments ($P < 0.05$). In the wheat season, the PD_whole_tree under the R-R treatment was significantly lower than that under the R-Fa treatment. On the whole, the microbial diversity in the wheat season was greater than that in the rice season, especially for fungi, with higher Observed_species, Shannon, Simpson, Chao1, ACE, and PD_whole_tree ($P < 0.01$).

Table 1 The alpha diversity of soil microbes under different cropping systems

Microbes	Seasons	Treatment	Observed_ species	Shannon	Simpson	Chao1	ACE	Goods_ coverage	PD_ whole_tree
Bacteria	Rice season	R-W	3374 a	9.654 a	0.996 a	3661 a	3715 a	0.990 a	259.0 a
		R-R	3430 a	9.589 a	0.996 a	4780 a	4067 a	0.986 a	265.3 a
		R-H	3388 a	9.583 a	0.995 a	3714 a	3771 a	0.989 a	262.0 a
		R-B	3301 a	9.567 a	0.995 a	3638 a	3674 a	0.989 a	252.9 a
		R-F	3406 a	9.631 a	0.996 a	3767 a	3805 a	0.989 a	263.7 a
		R-Fa	3403 a	9.622 a	0.995 a	3741 a	3785 a	0.989 a	262.9 a
	Winter season	R-W	3559 ab	9.786 a	0.997 a	4451 a	4220 a	0.985 a	244.0 ab
		R-R	3326 b	9.208 b	0.984 b	3666 a	3718 a	0.989 a	235.9 b
		R-H	3477 ab	9.833 a	0.997 a	3829 a	3881 a	0.989 a	243.7 ab
		R-B	3423 ab	9.766 a	0.997 a	3752 a	3818 a	0.989 a	234.2 b
		R-F	3585 ab	9.897 a	0.997 a	3957 a	4009 a	0.988 a	247.5 ab
		R-Fa	3675 a	9.958 a	0.997 a	4054 a	4108 a	0.988 a	257.9 a
Fungi	Rice season	R-W	469 ab	3.703 abc	0.781 a	515 a	517 ab	0.999 a	113.9 a
		R-R	465 ab	3.908 a	0.812 a	499 a	506 ab	0.999 a	107.9 ab
		R-H	469 ab	3.860 ab	0.809 a	517 a	515 ab	0.999 a	112.4 a
		R-B	488 a	3.700 abc	0.755 a	529 a	529 a	0.999 a	117.3 a
		R-F	437 ab	3.027 c	0.680 b	471 a	481 ab	0.999 a	106.3 ab
		R-Fa	352 b	3.078 bc	0.768 a	383 a	388 b	0.999 a	87.7 b
	Winter season	R-W	573 a	5.362 a	0.927 a	617 a	626 a	0.999 a	132.7 ab
		R-R	513 a	4.648 a	0.891 a	557 a	566 a	0.999 a	112.6 b
		R-H	539 a	5.121 a	0.936 a	586 a	591 a	0.999 a	126.1 ab
		R-B	552 a	5.277 a	0.933 a	598 a	597 a	0.999 a	130.0 ab
		R-F	522 a	4.696 a	0.902 a	676 a	610 a	0.998 a	120.8 ab
		R-Fa	582 a	5.335 a	0.938 a	626 a	630 a	0.999 a	143.3 a
Bacteria	Treatment		**	ns	ns	ns	ns	ns	**
	Season		ns	ns	ns	ns	ns	ns	ns
	Treatment $\times$ Season		ns	ns	ns	ns	ns	ns	ns
Fungi	Treatment		**	**	**	**	**	ns	**

Season	ns	ns	ns	ns	ns	ns	ns
Treatment $\times$ Season	ns	ns	ns	ns	ns	ns	*

Note: R-W, rice-wheat; R-R, rice-rape; R-H, rice-hairy vetch; R-B, rice-barley; R-F, Rice-faba bean; R-Fa, rice-fallow. Observed_ Species represent the number of intuitively observed species (i.e., the number of OTUs). Shannon means the total number of classifications in the sample and their proportion. The higher Shannon index indicated higher community diversity and a more uniform distribution of species. Simpson represents the diversity and uniformity of species distribution in the community. Chao1 estimates the total number of species included in the community sample. ACE estimates the number of OTUs in the community. Goods_ Coverage is the index of sequencing depth. PD_whole_Tree represents the genetic relationship of species in the community. Different lowercase letters in one column indicate significant differences among treatments ($P < 0.05$). ** represents significant difference at $P < 0.01$, * represents significant difference at $P < 0.05$, ns represents no significant difference ($P > 0.05$).

Fig. 1 shows the differences and similarities in the soil microbial community structure under different planting modes. The results of principal component analysis (PCA, Fig. 1) showed that PC1 and PC2 explained 18.25% and 15.57% of the variation in bacterial communities during the rice and winter seasons, respectively. For fungal communities, the values were 20.56% and 17.41% in both seasons. This indicates that the community structure is complex and cannot be fully described by one or two main factors. Although the overall explanatory power is low, we observed a certain trend in the winter season PCA plots. Microbial communities in the R-H, R-F, and R-Fa treatments were closer to each other. In contrast, those in R-W and R-B treatments were farther apart, with the R-R treatment showing clear separation from the others and clustering in the upper right quadrant.

Although the visual clustering is not obvious, statistical tests using Adonis (Table 2) indicated greater intergroup differences than intragroup differences, especially in the winter season. Significant differences in soil bacterial community structure between treatments R-W and R-H, R-H and R-F, R-H and R-Fa, and R-B and R-Fa in soil community structure were observed ($P < 0.01$), while significant differences between treatments R-W and R-R, R-H, and between treatments R-Fa and R-R, R-H, R-F were observed for the fungi ($P < 0.01$). The explanations of the various planting patterns for the difference in fungal community structure were greater than those for the difference in bacterial community structure, which were greater than 0.5 between treatments R-W and R-R, R-R and R-Fa.

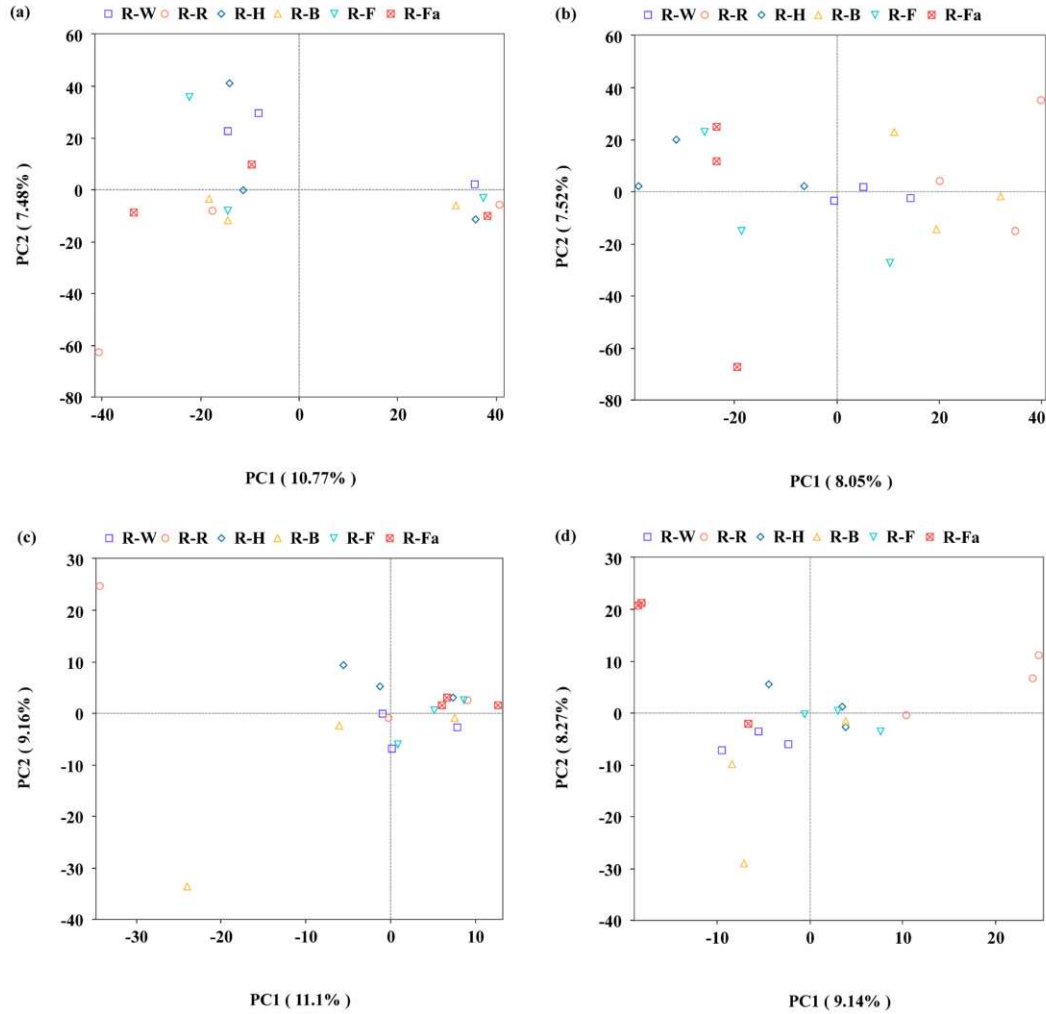


Fig. 1 The principal component analysis (PCA) of microbial community structure under different cropping systems
(a) Bacteria in the rice season; **(b)** Bacteria in the winter season; **(c)** Fungi in the rice season; **(d)** Fungi in the winter season. The X-axis and Y-axis represented the two selected main coordinate axes, and the percentage represented the main coordinate axis's explanation of the difference in sample composition; the X-axis and Y-axis scales were relative distances; points of different colors and shapes represented samples of different treatments, and the points with the same color and shape represented the three repetitions of the same treatment; the closer the two sample points were, the more similar the species composition of the two samples. R-W, rice-wheat; R-R, rice-rape; R-H, rice-hairy vetch; R-B, rice-barley; R-F, rice-faba bean; R-Fa, rice-fallow.

Table 2 Adonis analysis of soil microbial community structure in response to cropping systems

Microbes	Seasons	Treatments	R-W	R-R	R-H	R-B	R-F	R-Fa
Bacteria	Rice season	R-W	—					
		R-R	0.2188	—				
		R-H	0.1732	0.2083	—			
		R-B	0.1815	0.1552	0.1831	—		
		R-F	0.1506	0.1579	0.1377	0.1306	—	

		R-Fa	0.1387	0.1492	0.1362	0.1372	0.1181	—
		R-W	—					
		R-R	0.3095	—				
		R-H	0.2390*	0.3314	—			
	Winter season	R-B	0.219	0.2249	0.3121	—		
		R-F	0.2055	0.3099*	0.1960	0.2587	—	
		R-Fa	0.2059	0.2927*	0.1856	0.2585**	0.1697	—
		R-W	—					
		R-R	0.1449	—				
		R-H	0.1669	0.1053	—			
	Rice season	R-B	0.3271	0.3250	0.3670	—		
		R-F	0.1181	0.1683	0.1955	0.2962	—	
		R-Fa	0.3033	0.2456	0.3221**	0.5285**	0.2369	—
		R-W	—					
		R-R	0.5192*	—				
		R-H	0.4261*	0.4838	—			
	Winter season	R-B	0.2701	0.4390	0.4182**	—		
		R-F	0.4719	0.4457	0.3655	0.4615	—	
		R-Fa	0.2904*	0.5076*	0.3322**	0.3075	0.4633**	—

Note: The value in the table is R^2 , which represents the explanation of grouping to sample differences, that is, the ratio of group variance to total variance. Higher R^2 means greater explanation of grouping to differences. R-W, rice-wheat; R-R, rice-rape; R-H, rice-hairy vetch; R-B, rice-barley; R-F, rice-faba bean; R-Fa, rice-fallow. ** means $P < 0.01$.

Microbial community structure

Microbial community structure at the phylum level

The differences in microbial community structure at the phylum level across various planting modes were shown in Fig 2. The results showed that Proteobacteria was the dominant bacterial phylum in the rice and wheat seasons, accounting for 22.3%-25.6% of the soil bacterial community in this experiment (Fig 2a, b). The relative abundance of Unidentified_bacteria, Chloroflexi, and Acidobacter was lower than that of Proteobacteria, and the relative abundance of these four bacteria constituted more than 60% of the soil's total bacteria in the rice season. In the wheat season, the relative abundance of Proteobacteria, Unidentified_bacteria, Acidobacter, and Chloroflexi accounted for more than 60% of

the total bacteria in the soil, with *Acidobacter* showing a higher relative abundance than *Chloroflexi*. Analysis of between-group differences revealed no difference in key bacteria at the phylum level between the other treatments and R-W during the rice season. The relative abundance of the phylum *Bacteroidota* was greater in the R-H treatment than in the R-B treatment, and *Nitrospina* was more enriched in the R-Fa treatment compared to that in the R-F treatment (Table S2, $P < 0.05$). During the winter season, compared with rape planting, the relative abundance of the phyla *Bacteroidota* and *Planctomycetota* in the hairy vetch and fallow treatments were both significantly higher ($P < 0.05$). The relative abundance of *Myxococcota* in hairy vetch and faba bean treatments was significantly higher than that in barley treatment ($P < 0.05$).

The dominant phyla of soil fungi in this experiment were *Ascomycota* and *Mortierellomycota*, whose relative abundance accounted for 90.3%~93.6% of soil fungi in the rice season and 73.2%~90.5% in the wheat season (Fig 2c, d). *Ascomycota*, the most abundant phylum in rice soil, was observed to have the highest relative abundance in the R-Fa treatment, which was significantly higher than in R-W, R-H, and R-B (Table S2, $P < 0.05$). R-Fa had lower relative abundance of *Mortierellomycota* compared to R-W treatment ($P < 0.05$). R-B had higher *Mortierellomycota* abundance compared with R-R and R-Fa ($P < 0.05$). In addition, *Basidiomycota* was more abundant in R-W and R-R treatments than that in R-Fa ($P < 0.05$). During the winter season, *Ascomycota* was observed to have the lowest relative abundance in the R-W, while it was the most abundant in the R-R treatment ($P < 0.05$). By contrast, *Mortierellomycota* showed the opposite trend.

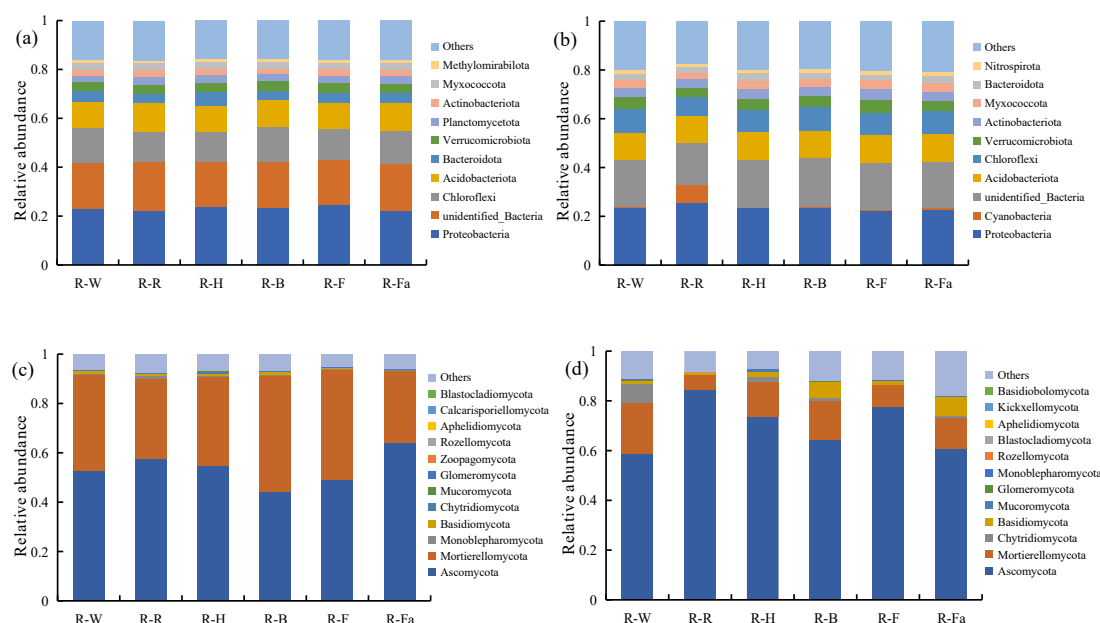


Fig. 2 The composition of the soil microbial community under different cropping systems at the phylum level (top 10)

(a) Bacteria in the rice season; (b) Bacteria in the winter season; (c) Fungi in the rice season; (d) Fungi in the winter season. R-W, rice-wheat; R-R, rice-rape; R-H, rice-hairy vetch; R-B, rice-barley; R-F, rice-faba bean; R-Fa, rice-fallow.

Microbial community structure at the genus level showed that dominant genera *UTCFXI*, *Sphingomonas* and *Luteimonas* accounted for about 10% of the total bacterial community in the rice season (Fig. 3a). During the winter season (Fig. 3b), *Unidentified_Chloroplast* and *Unidentified_Mitochondria* accounted for around 10.0% of the total bacterial community under the rice-rape treatment, while no obvious dominant bacterial genus was detected under the other treatments. In these treatments, the proportion of the top 10 genera was 13.4%, 12.3%, 13.8% and 12.7%, respectively, including *Rhodanobacter*, *Sphingomonas*, *UTCFXI*, *Candidatus_Solibacter*, and others. We found that the relative abundance of the dominant bacterial genus *UTCFXI* was the lowest in R-R treatment, significantly lower than that in R-W and R-B (Table S3, $P < 0.05$) during the rice season. Conversely, the relative abundance of *Rhodanobacter* under R-R treatment was the highest ($P < 0.05$). In the R-H treatment, *Luteimonas* was enriched, with a significantly higher relative abundance than in treatments R-W, R-R and R-Fa ($P < 0.05$). In addition, R-R treatment significantly increased the relative abundance of *Ellin6067* and *Nitrosospora*, and R-F treatment significantly increased the relative abundance of *Ellin6067* and *Terracidiphilus* in comparison with R-W treatment ($P < 0.05$). During the winter season, the relative abundance of *Rhodanobacter* under R-R treatment was highest, followed by R-B ($P < 0.05$). Compared to the R-W treatment, R-H, R-F and R-Fa significantly increased the relative abundance of *Haliangium*, R-H enriched *RB41*, and R-Fa enriched *Flavobacterium* ($P < 0.05$). The relative abundance of *Nitrosospora* was highest in R-B, followed by R-R and R-W ($P < 0.05$). Moreover, R-H enriched *Ellin6067* compared to the R-R and R-B treatments ($P < 0.05$).

Mortierella and *Torula* constituted about 60% of the total fungal community in the rice season (Fig. 3c), while the proportion of the top 10 genera in the total fungal community accounted for about 49.8% ~ 76.4% in winter season (Fig. 3d). In the treatment of rice - rape, rice - vetch and rice - faba bean, the dominant bacterial genera *Monographella* and *Bipolaris* accounted for 44.3%, 21.9% and 37.1% of the total fungal community, while the dominant fungi for the other treatments were *Mortierella* and *Ciliophora*, etc. Results of the metastat showed that *Mortierella* exhibited the highest relative abundance in the R-B treatment, which was significantly higher than in the R-R and R-Fa treatments ($P < 0.05$) during the rice season. However, the relative abundance of *Torula* was lowest in the R-B treatment, which was significantly lower than in the R-H and R-Fa treatments (Table S3, $P < 0.05$). Soil in the R-H treatment enriched the highest *Gibellulopsis* ($P < 0.05$). The relative abundance of *Pseudogymnoascus* in R-F was significantly higher than that in the R-H and R-Fa treatments ($P < 0.05$). In addition, the relative abundance of *Fusarium* in R-H was significantly higher compared to that in R-B and R-F treatments, *Gibberella* in R-R was significantly higher compared to that in R-W, R-B, and R-Fa treatments, while the relative abundance of *Fusicolla* in R-H was significantly higher than that in the R-Fa treatment ($P < 0.05$). During the winter season, R-R significantly enriched the *Monographella*, followed by treatments with R-B and R-H, with higher relative abundance compared to the other treatments ($P < 0.05$). The same trend was observed in *Bipolaris*, with R-R and R-F both significantly increasing its relative abundance compared to R-W, R-B, or R-Fa ($P < 0.05$). On the contrary, the relative abundance of *Mortierella* in R-W, R-B, and R-Fa was significantly higher than that in R-R, R-H or R-F treatments ($P < 0.05$). The R-H treatment enriched the highest *Gibellulopsis*, while the R-R treatment enriched the highest *Penicillium* compared to other treatments ($P < 0.05$). The relative abundance of *Fusarium* in R-H and R-Fa was significantly higher compared to the other

treatments ($P < 0.05$). Moreover, compared to R-W, R-R, R-H, R-F, and R-Fa significantly decreased the relative abundance of *Blumeria*, while R-B and R-Fa treatments significantly increased the relative abundance of *Gibberella* ($P < 0.05$).

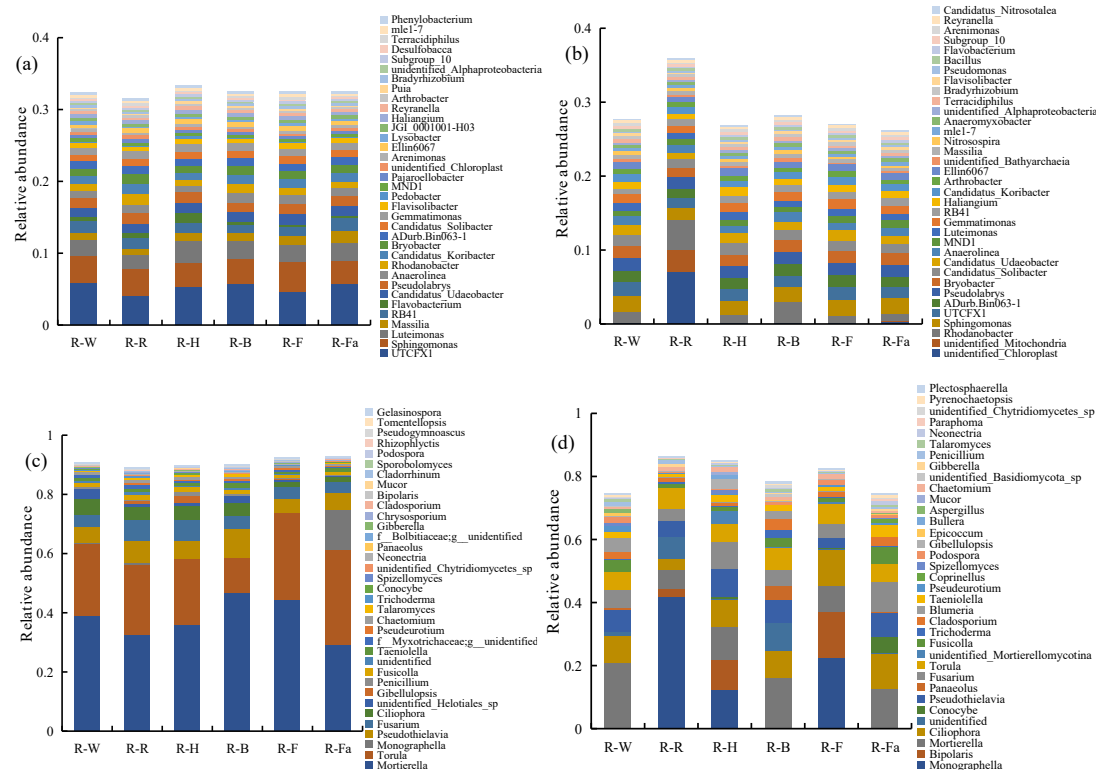


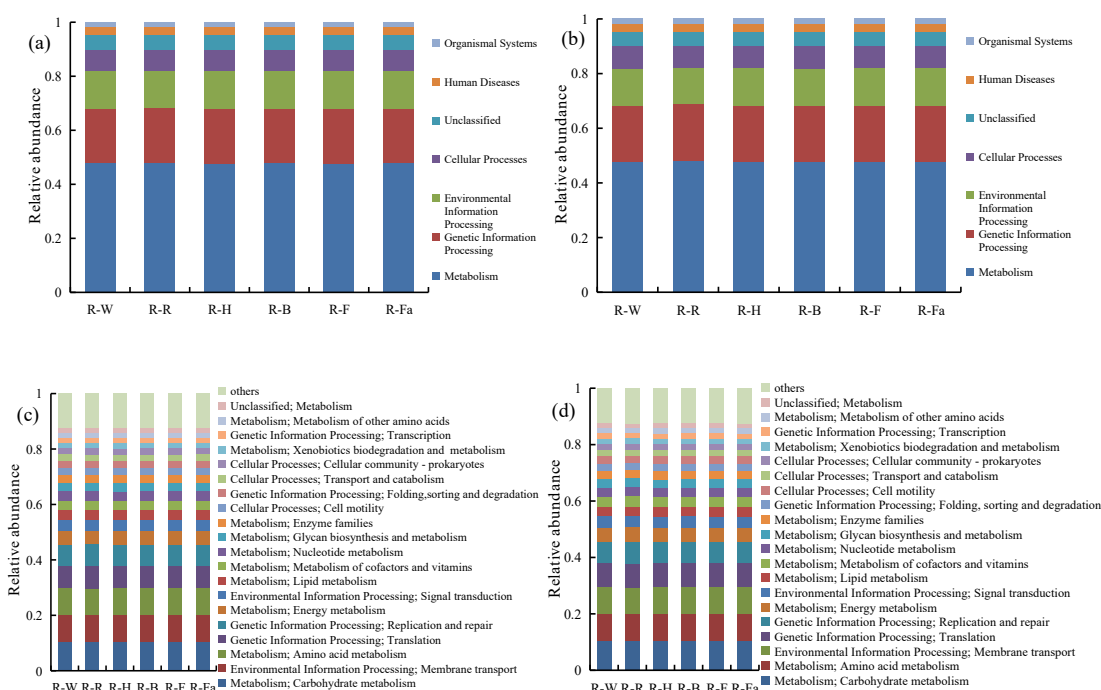
Fig. 3 The composition of the soil microbial community under different cropping systems at the genus level (top 35) (a) Bacteria in the rice season; (b) Bacteria in the winter season; (c) Fungi in the rice season; (d) Fungi in the winter season. R-W, rice-wheat; R-R, rice-rape; R-H, rice-hairy vetch; R-B, rice-barley; R-F, rice-faba bean; R-Fa, rice-fallow.

Functional prediction

As shown in Fig. 4, there were differences in the relative functional abundance of soil bacteria and fungi under different planting modes. The bacterial functions mainly include metabolism, genetic_information_processing, environmental_information_processing, cellular processes, human_diseases, and organismal_systems in level 1 (Fig. 4a, b). The relative abundance of metabolism in R-Fa was significantly higher than that in R-H treatment during the rice season, which was higher in R-F compared to that in R-B treatment during the wheat season (Table S4, $P < 0.05$). As for level 2, the top three dominant functional groups were Metabolism-Carbohydrate metabolism, Environmental Information Processing-Membrane transport, and Metabolism-Amino acid metabolism, the sum of whom was approximately 30% (Fig. 4c, d). Compared with the R-W treatment, treatments of R-R improved the abundance of Genetic Information Processing-Transcription during the rice season (Table S4, $P < 0.05$). During the wheat season, compared with the R-W treatment, R-R improved the relative abundance of Metabolism-Biosynthesis of other secondary metabolites; R-H decreased the relative abundance of Metabolism-Energy metabolism, Cellular Processes-Cellular community - prokaryotes, while increasing the abundance of Genetic Information Processing-Transcription; R-B decreased the

abundance of Organismal Systems-Endocrine system; R-F increased the abundance of Genetic Information Processing-Folding, sorting and degradation; and R-Fa increased the abundance of Cellular Processes-Transport and catabolism as well as Genetic Information Processing-Transcription ($P < 0.05$). Compared with R-B treatment, R-H increased the abundance of Metabolism-Carbohydrate metabolism, Metabolism-Enzyme families and Genetic Information Processing-Transcription; R-F increased the abundance of Metabolism-Enzyme families and Genetic Information Processing-Folding, sorting and degradation ($P < 0.05$). Additionally, R-H increased the abundance of Metabolism-Enzyme families more than that in R-R, and increased Metabolism-Lipid metabolism and Metabolism-Metabolism of terpenoids and polyketides than those in R-Fa, while R-F increased the abundance of Metabolism-Lipid metabolism, Metabolism-Nucleotide metabolism and Cellular Processes-Transport and catabolism than those in R-Fa ($P < 0.05$).

In this study, 74.6%~86.0% of fungal OTUs in rice seasons and 65.3%~87.1% in wheat seasons were successfully assigned to nutritional types, including Saprotroph, Symbiotroph, Pathotroph and mixed function types (Fig. 4e, f, $P < 0.05$). During the rice season, Undefined_Saprotroph was the predominant function, whose relative abundance was highest in the R-B treatment and lowest in the R-Fa treatment, while the second most abundant Endophyte-Plant_Pathogen-Wood_Saprotroph was in the opposite trend (Table S4, $P < 0.05$). In addition, the relative abundance of Plant_Pathogen was significantly increased in R-H, compared to those in the R-W, R-B and R-F planting modes ($P < 0.05$). During the wheat season, Plant_Pathogen was the most abundant functional type, with the higher relative abundance in R-R, R-H and R-F treatments than those in R-W, R-B and R-Fa treatments ($P < 0.05$). Compared to R-W treatment, the relative abundance of Undefined_Saprotroph was significantly decreased in R-H and R-F treatments ($P < 0.05$). R-Fa treatment enriched the most Plant_Pathogen-Soil_Saprotroph-Wood_Saprotroph than the others ($P < 0.05$). The relative abundance of Endophyte-Plant_Pathogen- Wood_Saprotroph in R-R was significantly higher compared to those in R-H and R-Fa, while Dung_Saprotroph-Endophyte- Litter_Saprotroph-Undefined_Saprotroph in R-R was lower than those in R-H and R-Fa ($P < 0.05$).



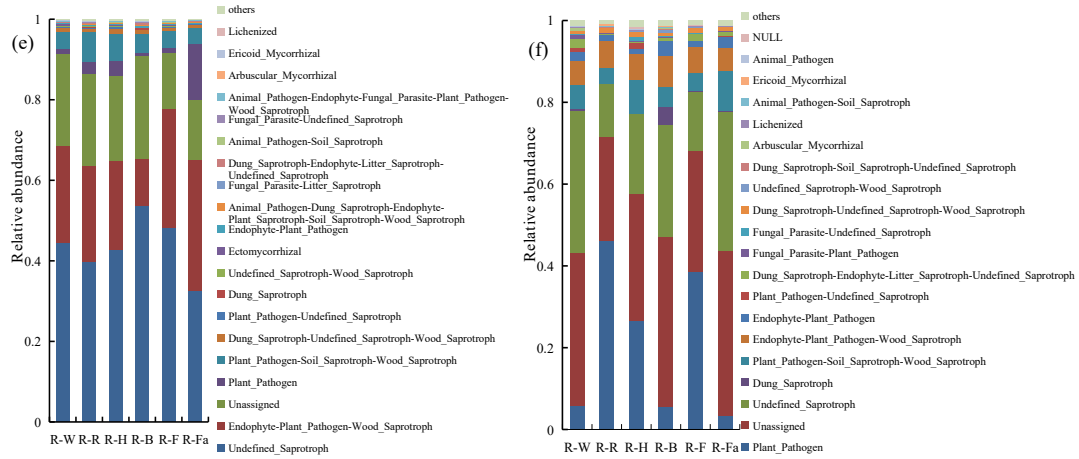


Fig. 4 Relative abundance of functional annotation of bacteria (Tax4Fun) and fungi (FUNGuild) under different cropping systems. (a) Bacteria in the rice season (level 1); (b) Bacteria in the winter season (level 1); (c) Bacteria in the rice season (level 2); (d) Bacteria in the winter season (level 2); (e) Fungi in the rice season; (f) Fungi in the winter season. R-W, rice-wheat; R-R, rice-rape; R-H, rice-hairy vetch; R-B, rice-barley; R-F, rice-faba bean; R-Fa, rice-fallow.

Correlation analysis

The canonical correlation analysis (Fig. 5) of environmental factors and soil microbial abundance showed that the tested soil physical and chemical properties could explain 47.3% and 66.2% of the difference in bacterial community structure in the rice and wheat seasons, respectively, while the interpretation degree of the difference in fungal community structure is 49.3% and 48.5%, respectively. The bacterial sample points were primarily concentrated on the right side of the first axis, whereas the fungal samples exhibited the opposite distribution. In the rice season, there was a significant correlation between soil pH, STC, STN, and bacterial community ($P < 0.01$). A significant negative correlation between soil NO_3^- -N and NH_4^+ -N content ($P < 0.01$) with the bacterial community was observed in the wheat season ($P < 0.05$). As for the fungal community, it was significantly correlated to soil NO_3^- -N, STN, STC, BD, and SOC content in the rice season ($P < 0.05$). In the wheat season, a significant correlation between soil NO_3^- -N, NH_4^+ -N content ($P < 0.01$) and BD ($P < 0.05$) with the fungal community was observed.

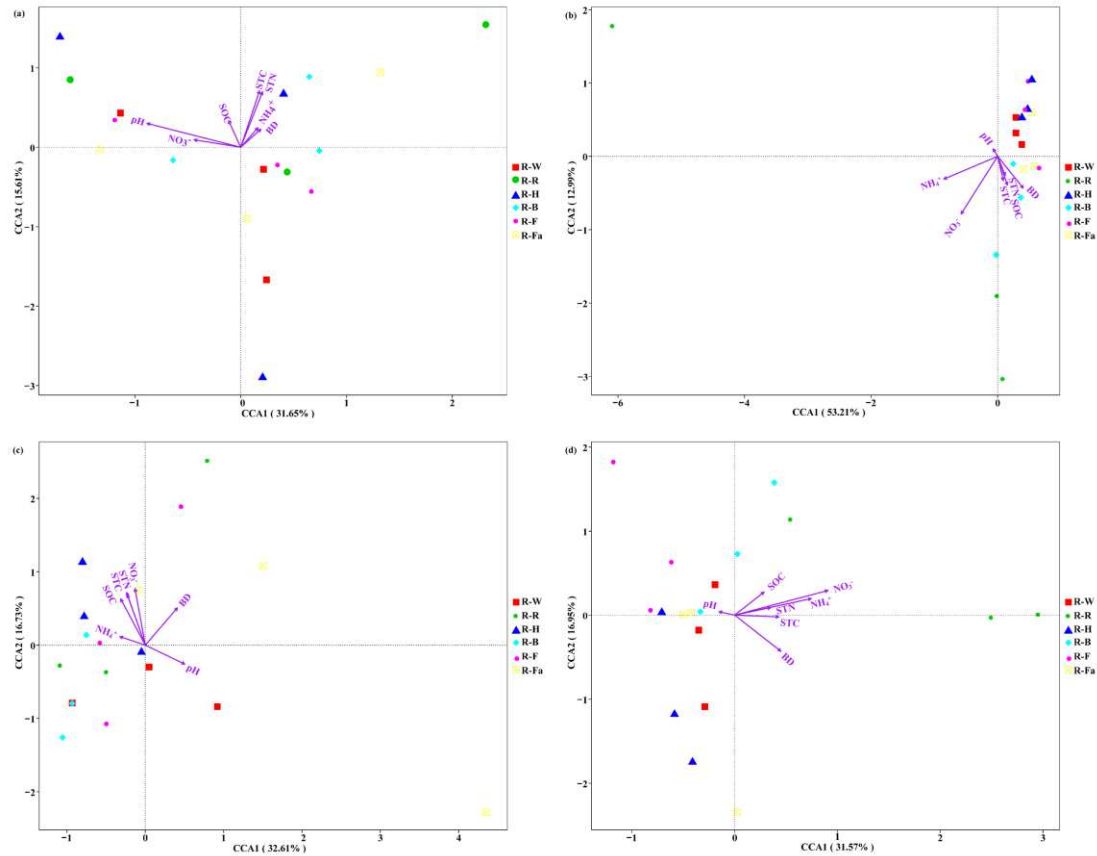


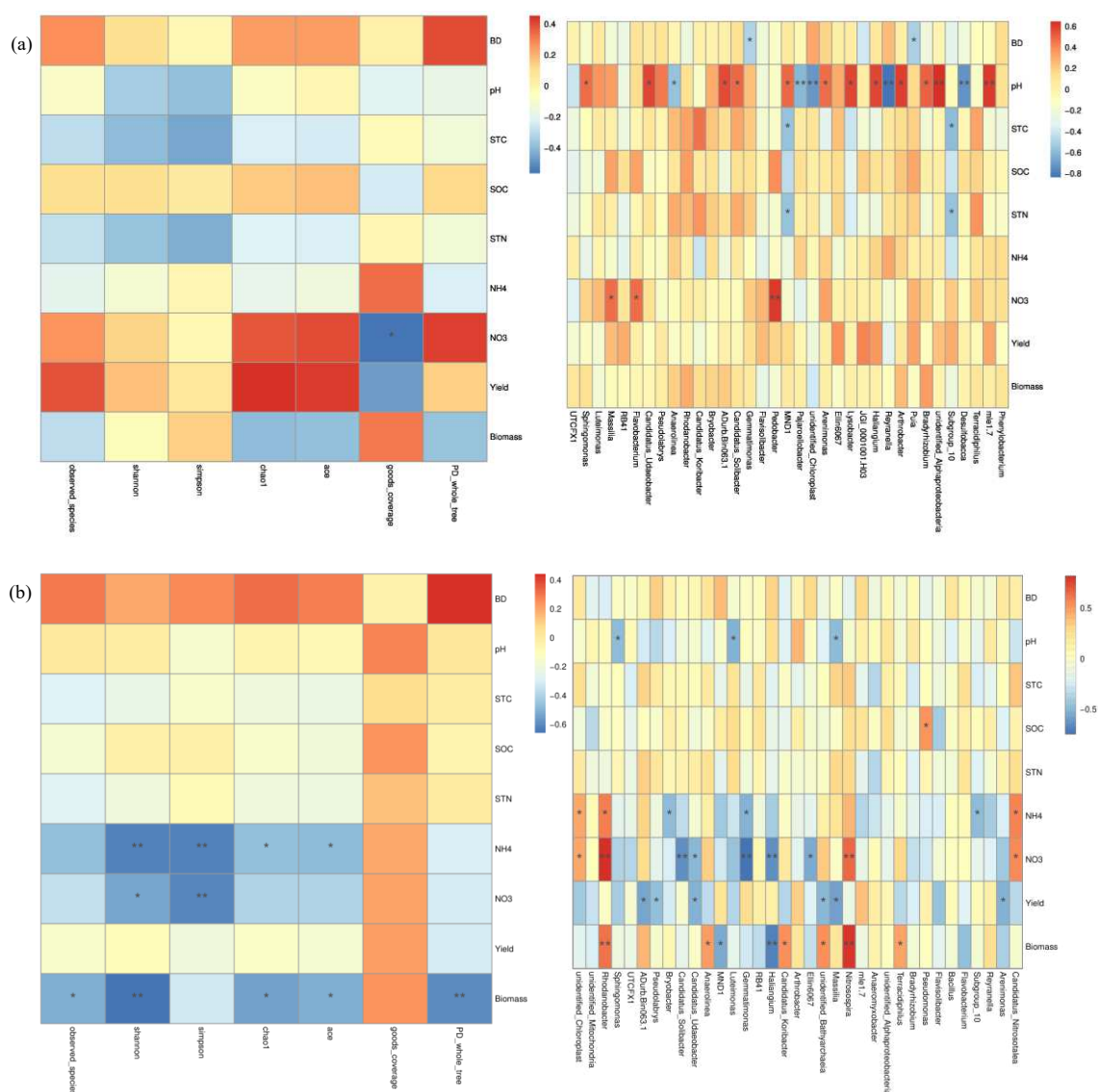
Fig. 5 Canonical correlation analysis (CCA) of the soil microbial abundance and environmental factors under different cropping systems
(a) Bacteria in the rice season; (b) Bacteria in the winter season; (c) Fungi in the rice season; (d) Fungi in the winter season. R-W, rice-wheat; R-R, rice-rape; R-H, rice-hairy vetch; R-B, rice-barley; R-F, rice-faba bean; R-Fa, rice-fallow.

The correlation between environmental factors and α diversity and abundance at the genus level (top 35) of soil bacterial (a, b) and fungal (c, d) communities is shown in Fig. 6. The results showed that there was a significant negative correlation ($P < 0.05$) between the good_coverage of bacteria and the soil NO₃⁻-N content in the rice season. In the winter season, the bacterial diversity was negatively correlated with soil NH₄⁺-N and NO₃⁻-N content ($P < 0.05$). As for fungi, their diversity was negatively correlated with soil pH during the rice season, while negatively correlated with soil NH₄⁺-N in the winter season ($P < 0.05$).

Most of the top bacterial and fungal genus abundance was positively correlated with STC, SOC, and STN, especially *Chaetomium* and *Rhizophlyctis* in the rice season ($P < 0.05$). In contrast, *Panaeolus* and *Spizellomyces* were negatively correlated with STC, SOC, and STN in the winter season ($P < 0.05$). Bacterial genera regulating N cycling, such as *Massilia*, *Flavobacterium*, and *Pedobacter* in the rice season and *Rhodanobacter*, *Nitrosospira*, *Candidatus_Nitrosotalea* in the winter season, were positively correlated with NH₄⁺-N and NO₃⁻-N ($P < 0.05$). In the rice season, the relative abundance of most top bacterial genera was positively correlated with soil pH, while most of the top fungal genera were negatively correlated with soil pH ($P < 0.05$).

During the winter season, the bacterial diversity was significantly negatively correlated to the

annual biomass ($P < 0.05$). A negative correlation between rice yield and the relative abundance of *ADurb.Bin063.1*, *Pseudolabrys*, *Candidatus-Udaeobacter*, *unidentified_Bathyarchaeia*, *Massilia*, and *Arenimonas* in the winter season were observed ($P < 0.05$). *Rhodanobacter*, *Anaerolinea*, *Candidatus_Koribacte*, *unidentified_Bathyarchaeia*, *Nitrospira*, and *Terracidiphilus* were significantly positively correlated with annual biomass, while *MND1* and *Haliangium* were negatively correlated with annual biomass. The relative abundance of fungi *Gibberella* in the rice season and *Ciliophora*, *Coprinellus* in the winter season was significantly positively correlated with rice yield, while *Fusicolla* was the opposite. *Unidentified_Helotiales_sp* in the rice season and *Blumeria*, *unidentified_Basidiomycota_sp* in the winter season were significantly positively correlated with annual biomass, while *Fusarium* was negatively correlated with annual biomass.



crops, which provide diverse C sources and alter the soil environment, thereby impacting microbial diversity (Cline et al., 2018; Song et al., 2018). This study systematically elucidated the shaping effects of different winter cultivation patterns on soil microbial community structure by integrating α - and β -diversity analyses. Firstly, species coverage across all samples exceeded 98%, indicating that the sequencing depth was sufficient to capture genuine differences in diversity among treatments, thereby providing a reliable data foundation for subsequent statistical analyses (Gihring et al., 2012). We observed a decline in bacterial and fungal diversity in the R-R treatment (Table 1). This may be due to the selective inhibition of specific microbial groups by root secretions of cruciferous plants (like glucosinolates) (Bernard et al., 2012; Bressan et al., 2009), leading to lower overall diversity. The treatment of leguminous crop planting in winter (i.e., R-H, R-F) tended to increase bacterial diversity but reduce fungal diversity, while the effect was not significant. As was reported by a meta-analysis, introducing legumes to rotation had no consistent effects on soil microbial diversity or richness, where some grain-legume rotations increased microbial richness while others decreased microbial richness (Venter et al., 2016). Interestingly, we found the microbial diversity in the winter season was greater than that in the rice season, while fungi responded more strongly to seasonal changes than bacteria (Table 1). The aerobic conditions of the uplands increase microbial and enzyme activities, according to the concept that well-aerated soils offer a more diverse array of niches, supporting a broader spectrum of microbial taxa (Hartmann and Six, 2022; Li et al., 2024). In contrast, the anaerobic environments and intensive environmental changes lead to harsh selection of microbial communities (Liesack et al., 2000; Lu et al., 2018), thereby reducing microbial diversity in paddy soils. The reason for the difference in response between bacteria and fungi may be that fungi are more sensitive to soil water and aeration, because most of them are aerobic organisms and grow slowly, exhibiting stronger dependence on host plants and higher specificity in C source utilization (Boyle et al., 2024).

The β -diversity analysis (Fig. 1) revealed that PCA provided a limited explanation for the variation in bacterial (33.82%) and fungal (37.97%) communities, indicating that microbial community composition is driven by multiple factors. However, the Adonis test (Table 2) confirmed that crop type is the key driver of microbial community differentiation, with fungal communities demonstrating greater sensitivity to planting patterns. The PCA ranking plot reveals significant differences in bacterial communities among leguminous crops (R-H, R-F), graminaceous crops (R-W, R-B), and fallow land (R-Fa). This confirms that leguminous crops alter the soil C/N through symbiotic N fixation, thereby selecting specific bacterial taxa with distinct ecological functions (Carlsson et al., 2003; Yang et al., 2021). Furthermore, the R-R treatment exhibited a unique separation trend, corroborating the reduction in α -diversity under this planting mode. In conclusion, the differentiation in winter crop types drives the formation of bacterial and fungal communities through dual mechanisms of species selection and resource regulation, providing a theoretical basis for optimizing crop rotation systems to precisely regulate soil microbial ecological functions.

Effects of planting patterns on the microbial community structure and function

This study conducted a systematic analysis of soil microbial communities under different cropping systems during the rice growth season and winter. It elucidated the regulatory effects of planting regimes on soil microbial community structure at the phylum and genus levels. The results demonstrated that planting regimes drive differentiated responses in bacterial and fungal communities. These effects occur through crop-type-specific root secretions, stubble characteristics, and changes in

the soil microenvironment, and they exhibited distinct seasonal dynamics. Analysis at the phylum level revealed that the most abundant bacterial and fungal phyla identified are Proteobacteria and Ascomycota (Fig. 2a, b), which are consistent with other research on agricultural systems in China farmland (Liu et al., 2023; Zong et al., 2024). The rice-legume system (R-H) enriched nutrient-rich bacteria involved in the C and N cycles, including Bacteroidota and Planctomycetota (Buchan et al., 2014; Lage et al., 2025), as well as the predatory Myxococcota phylum (Zhang et al., 2023). Further analysis at the genus level revealed that legume rotation systems (R-H, R-F) enriched acidophilic bacteria (Fig. 3a, b), such as *Ellin6067*, *Terracidiphilus*, and *RB41* associated with organic matter decomposition (Garcia-Fraile et al., 2016; Stone et al., 2021; Xia et al., 2005). The predatory bacterium *Haliangium* was also enriched (Zhang et al., 2023). This enrichment is primarily attributed to the low C/N ratio in legume plant tissues, which release abundant easily decomposable organic C and N compounds during decomposition (Frey et al., 2004; Mitchell et al., 2016). These compounds providing efficient energy sources for eutrophic bacterial communities. The results also suggested that the rhizosphere microenvironment of legume crops facilitates the complexity of microbial interaction networks. The R-R treatment significantly enriched the denitrifying bacterial genus *Rhodanobacter* (Cao et al., 2021), suggesting that these bacteria may be tolerant to rape root secretions, thereby gaining a niche advantage. Enrichment of the *Flavobacterium* genus in the R-Fa indicates that this cultivation approach may have potential for preventing soil-borne diseases (Su et al., 2021). Furthermore, *Nitrosospora* exhibited the highest abundance in the R-B treatment and maintained elevated levels in R-W, indicating that crop rotation involving graminaceous crops is conducive to sustaining soil ammonia-oxidizing microbial activity (Shi et al., 2018).

Regarding fungal communities, phylum-level analysis revealed that Ascomycota exhibited the highest abundance under the R-Fa treatment during the rice season, whereas Basidiomycota showed greater dominance under continuous crop cultivation (Fig. 2c, d). Furthermore, Ascomycota was observed to have the highest relative abundance in the R-Fa treatment in rice soil, and it was the most abundant in the R-R treatment in the winter season. These results reflect the distinct response strategies of fungal communities to seasonal variations and crop residue inputs. Moving to the genus-level analysis, pronounced disease-associated variations were further revealed (Fig. 3c, d). Specifically, cropping systems involving rapeseed (R-R) and legume crops (R-H, R-F) significantly enriched phytopathogenic fungal genera, including *Gibberella* (Zhai et al., 2026), *Bipolaris* (Wang et al., 2024), and *Fusarium* (Ma et al., 2013). Thus, the findings indicate that although rotations of cruciferous and leguminous crops improve soil fertility, they may elevate ecological risks of specific soil-borne diseases. In contrast, graminaceous crop rotations (R-W, R-B) and R-Fa facilitated the preservation of saprophytic beneficial fungi, such as *Mortierella*, which play critical roles in organic matter decomposition, nutrient activation, and pathogen suppression (Hu et al., 2026).

Functional prediction demonstrated that planting patterns have effects on the structure and function of soil microbial communities, which exhibit strong coupling characteristics (Fig. 4). For instance, planting rape in the winter season (R-R) enhanced the biosynthesis of secondary metabolites, and markedly elevated the abundance of transcription-related functions during the rice growing season. Combined with the enrichment of *Rhodanobacter* and *Nitrosospora* under R-R at the genus level (Fig. 3), this suggests that rape may regulate the expression of functional genes associated with N cycling by modulating microbial transcriptional activity and secondary metabolism. Planting hairy vetch (R-H) significantly increased the functions of transcription, carbohydrate metabolism, and enzyme families, while planting faba beans (R-F) increased the functions related to enzyme families as well as folding,

sorting and degradation, which further verifies that legume rotation facilitated C-degrading enzyme systems and protein turnover-related biological processes. Moreover, the treatments R-R, R-H and R-F raised the abundance of plant pathogen-related functions in the wheat season (Fig. 4f). This result is highly consistent with the enrichment of potential pathogenic fungi, including *Fusarium* and *Bipolaris* at the genus level (Fig. 3c, d). This indicates that cruciferous and leguminous crops may increase ecological risks of soil-borne diseases, and the enrichment of pathogenic fungal functional groups demonstrates cross-season persistence. Overall, the optimization of planting regimes should comprehensively consider the differential regulatory effects of crop species on distinct bacterial and fungal functional groups. It is necessary to improve soil nutrient utilization efficiency while establishing ecological capacity for the prevention and control of soil diseases, so as to achieve sustainable agricultural production.

The relationships between soil properties, microbial community composition, and crop productivities

The structure and diversity of soil microbial communities are influenced by various factors, such as soil physicochemical properties, the quantity and quality of substrate; this influence varies depending on climate, soil type, and other factors (Feng et al., 2009; Hu et al., 2020; Labouyrie et al., 2024). Soil physical and chemical properties are the key factors driving microbial growth and activity (Muhammad et al., 2014). In particular, changes in nutrient availability directly affect the structure and abundance of soil microbial communities, which function as ecological filters by selecting specific functional groups (Yu et al., 2021). In this study, although soil physicochemical properties such as bulk density, total C, and total N did not differ statistically between treatments, CCA ranking and Spearman analysis revealed that they were significant environmental factors driving microbial community structural differentiation (Fig. 5, 6). This reflects the sensitivity of microbial niche differentiation to changes in the environment. The analysis results also revealed that *Rhizophlyctis* (Lange et al., 2019), a core microbial group involved in organic matter degradation, showed a significantly positive correlation with soil C and N contents. This indicates that fungal-driven saprophytic food webs are more active in microhabitats characterized by relatively high organic matter levels. Environments rich in C and N supply sufficient substrates for these decomposers, enabling them to gain competitive advantages over bacteria. Both pivotal N-cycling genera, *Rhodanobacter* and *Nitrosospora*, displayed significant positive correlations with soil ammonium N and nitrate N, revealing their key roles in the N transformation network. *Rhodanobacter* is not only linked to nitrate N via denitrification, but also potentially participates in the Feammox process (Zhang et al., 2024b). As typical ammonia-oxidizing bacteria (Lao et al., 2025), *Nitrosospora* positively correlates with ammonium N due to its dependence on ammonia substrates. At the same time, its positive relationship with nitrate N reflects the accumulation of cascade products from complete nitrification at sites with intense nitrification activity. The simultaneous associations of these two genera with both N forms highlight their core ecological functions in connecting diverse soil N pools and driving N transformation.

Previous studies generally believe that pH is a key factor in regulating microbial community structure (Rousk et al., 2010; Wang et al., 2019). In this study, a positive correlation between pH and the most dominant bacterial genera was observed during the rice season, whereas the opposite was observed for fungi (Fig. 6), consistent with conclusions drawn from most studies (Duan et al., 2025). However, as being in a near-neutral environment, the microbial differentiation observed may not be attributed to acid-base stress in the traditional sense. On the one hand, microbial groups in the study

site might possess narrow optimal pH ranges. Thus, even slight pH fluctuations can significantly alter their relative abundance. On the other hand, pH variation could indirectly regulate microbial metabolism by changing the availability of soil trace elements, including iron, manganese, copper, and zinc (Dai et al., 2023). This result suggests that evaluating microbial responses to farmland management practices should not merely focus on pH stress thresholds. Subtle pH fluctuations within neutral ranges also carry profound ecological implications for trace element availability, C balance, and nutrient cycling.

Furthermore, our results revealed a significant negative correlation between soil bacterial diversity in winter and annual biomass, which may reflect resource competition and functional redundancy within specific agroecosystems (Louca et al., 2018; Van Der Heijden et al., 2008). At the level of key microbial taxa, bacterial taxa including *Rhodanobacter*, *Nitrosospora*, and *Terracidiphilus* in winter soils were positively correlated with annual biomass. These genera enhanced both C and N cycling efficiency and improved soil nutrient availability, thereby directly facilitating the accumulation of total biomass within the ecosystem (Cao et al., 2021; Garcia-Fraile et al., 2016; Lao et al., 2025). The relative abundance of *Gibberella* in the rice growing season and *Coprinellus* in winter showed positive correlations with crop yield. They may promote crop growth by secreting hormones like gibberellin or accelerating nutrient mineralization (Fabros et al., 2025; Shani et al., 2024). In addition, *Unidentified_Helotiales_sp* detected in the rice season, as well as *Blumeria* and *unidentified_Basidiomycota_sp* in winter, were also positively associated with annual biomass, further demonstrating the beneficial roles of specific saprotrophic fungi in year-round nutrient cycling. On the contrary, genera such as *Pseudolabrys*, *Candidatus-Udaeobacter*, and *Arenimonas* were negatively correlated with rice yield. As oligotrophic microorganisms, they may be outcompeted by beneficial microbes in high-yield paddy fields with balanced nutrients and vigorous root activity. Moreover, *Fusicolla* and *Fusarium* exhibited consistently negative correlations with yield and annual biomass across seasons, possibly acting as key biological constraints on crop productivity. Overall, microbial communities exerted season-specific effects and displayed functional differentiation on rice yield and biomass. In particular, the persistent adverse impacts of *Fusarium* highlight the necessity of targeting the suppression of pathogenic fungi and enriching beneficial functional microbes in this cropping system.

Limitations and future perspectives

Despite the comprehensive insights gained from this study, several limitations should be acknowledged. First, this investigation was conducted at a single experimental site, which may constrain the generalizability of the findings across diverse agroecological regions with varying soil types and climatic conditions. Second, the functional predictions for bacteria (derived from Tax4Fun) and for fungal trophic modes (derived from FUNGuild) provide indirect inferences rather than direct functional validation through metagenomic, metatranscriptomic, or enzymatic assays. Moreover, the correlative nature of the analyses (the Spearman correlation and CCA) does not establish causality, and the absence of manipulative experiments (e.g., inoculation of keystone taxa) limits definitive attribution of functional contributions. Future research should prioritize long-term and multi-site field experiments to validate the observed patterns across broader spatial and temporal scales. The integration of multiomics approaches—including metagenomics, metatranscriptomics, and metabolomics—will enable direct elucidation of functional gene expression, metabolic pathways, and strain-level functional

differentiation. Additionally, high-frequency sampling across entire crop growth seasons would further resolve temporal dynamics and microbial successional patterns. Such multidimensional and mechanistic approaches will advance our understanding of microbiome-mediated rotation effects and provide a robust scientific foundation for designing sustainable cropping systems that harmonize soil health, disease suppression, and crop productivity.

Conclusion

Comprehensive results demonstrated that seasonal fluctuations and rotation patterns synergistically regulated the community structure, diversity, and ecological functions of soil microorganisms in paddy-upland fields. Fungal communities exhibited a higher sensitivity to external environmental changes compared to bacterial communities, whereas overall soil microbial diversity was greater in winter than during the rice growing season. Planting rape and fallow in the winter season reduced soil microbial diversity to varying degrees. Different rotation strategies selectively enriched unique, dominant bacterial and fungal taxa and reshaped microbial functional pathways (e.g., carbohydrate metabolism and genetic information processing), as well as fungal trophic modes. Rice-rape and rice-legume rotations effectively optimized soil microbial metabolic potential but simultaneously increased the accumulation of phytopathogenic fungi. Soil pH, soil organic C, total N, nitrate N, and ammonium N served as pivotal environmental factors mediating microbial community assembly under varying rotation regimes. The abundance of N-cycling functional microbiota was tightly linked to soil inorganic N concentrations, while specific microbial taxa directly determined crop biomass and rice yield. Collectively, post-rice legume rotation and rape rotation offer distinct advantages in modulating beneficial microbial populations and regulating microbial functions. We recommend the rational adoption of rice-legume (e.g., hairy vetch and faba bean) and rice-rape rotations in the paddy-upland double cropping regions of southern China. When combined with targeted disease management, these rotation practices can achieve the dual goals of sustainable soil health and stable crop productivity.

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Declarations

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Author Contributions

Liuge Wu: Writing—original draft, Formal analysis, Data curation. Tao Chen: Writing—original draft, Writing—review & editing, Data curation, Funding acquisition. Shenji Yan: Investigation, Formal analysis, Data curation. Aixing Deng: Investigation, Formal analysis, Data curation. Xiaosha Li: Investigation, Formal analysis. Jun Zhang: Supervision, Investigation, Validation. Chengyan Zheng: Supervision, Investigation, Validation. Ziyin Shang: Software, Methodology. Xin Zhang: Software, Methodology, Funding acquisition, Writing—review & editing, Supervision, Conceptualization. Zhenwei Song: Funding acquisition, Writing—review & editing, Supervision, Conceptualization. Weijian Zhang: Funding acquisition, Writing—review & editing, Supervision, Conceptualization.

Data availability

Data will be made available on request.

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