CHILEAN JOURNAL
of AGRICULTURAL RESEARCH
RESEARCH ARTICLE

Responses of soil microbial community structure and diversity to different cropping patterns of rice

Binjuan Yang[1], Yao Huang[1], Jiaxin Yuan[1], Qiliang Hu[1], Guoqin Huang[1*], Muhammad Umair Hassan[1], Tahani A.Y. Asseri[2], and Mohamed Hashem[3]

[1]Jiangxi Agricultural University, Research Center on Ecological Science, School of Agricultural Sciences, Nanchang 330045, China.
[2]King Khalid University, College of Science, Department of Biology, Abha 61413, Saudi Arabia.
[3]Assiut University, Faculty of Science, Department of Botany and Microbiology, Assuit 71516, Egypt.
[*]Corresponding author (hgqmail441@sohu.com)
Received: 28 May 2025; Accepted: 28 August 2025, doi:10.4067/S0718-58392025000600835

ABSTRACT

Soil microbes play an important role in nutrient cycling, and their richness and diversity are greatly influenced by cropping patterns and soil management systems. However, the effect of different cropping systems of rice on soil microorganism is still obscure. Therefore, this field experiment was conducted to study the effects of various paddy-upland multiple cropping rotation patterns on soil microbial community structure and diversity. The experiment comprised different treatments, Chinese milk vetch (*Astragalus sinicus* L.)-double cropping rice (CRR, CK), rape-early rice-late rice (RRR), potato (*Solanum tuberosum* L.)-early rice-late rice (PRR), Chinese milk vetch-early rice-sweet potato (*Ipomoea batatas* (L.) Lam.) late soybean (*Glycine max* (L.) Merr.) (CRI), and rape (*Brassica napus* L.)-early rice-sweet potato late soybean (RRI). The results showed that rapeseed and Chinese milk vetch in winter was conducive to increasing bacterial community richness and diversity. For each cropping pattern, the top three dominant phyla in paddy fields were *Proteobacteria*, *Chloroflexi* and *Actinobacteria* in terms of relative abundance. The community structure of soil bacteria showed more significant internal variability in CRI and RRI treatments and less internal variability in others treatments. Redundancy analysis of soil bacterial community structure and soil chemical properties revealed that soil bacterial community structure changes were primarily influenced by organic C, its fractions, and N content. In conclusion, Chinese milk vetch-early rice-sweet potato and late soybean cropping patterns may be considered for sustainable early and late rice production due to their beneficial impacts on soil bacterial abundance, diversity, and soil properties.

Key words: Bacterial diversity, community structure, cropping rotation, paddy field soil carbon.

INTRODUCTION

Soil is an important source of crop production, and crops and soil are linked with each other and mutually affect the each other (Padalia et al., 2018). Soil nutrient availability regulates the impact of soil microbes on plant performance, and nutrient dynamics in soil are largely impacted by agricultural practices (Bargali et al., 2019). Soil microorganisms are important indicators of soil variability, accumulation and degradation of soil organic matter (SOM) and they also contribute to maintain the nutrient cycling and soil fertility (Zhang et al., 2005). The growth of soil microbes is greatly affected by exogenous additives, soil nutrients, soil pH, texture, climatic conditions, vegetation types, tillage practices, and cropping patterns (Chen et al., 2024). Soil microbial community structure and diversity are closely related to interact with the soil ecosystem environment, and different factors likes cropping systems, field management practices and environmental conditions can affect soil microbial community structure and diversity (Xu et al., 2019). Various agricultural practices and cropping systems affect soil physico-chemical properties, which in turn impact microbial community structure (Xu et al., 2020).

During the last five decades farmers have been using intensive agriculture practices which are negatively affecting crop productivity, soil quality, and climate (Bedolla-Rivera et al., 2023). Continuous cropping is a widely used practice globally; however, it negatively affects plant growth and weakens photosynthesis, reduces plant resistance, and decreases yield and quality (Ma et al., 2023). Continuous cropping cause nutrient imbalance, increases the accretion of toxic allelochemicals, increases the abundance of soil-borne pathogens, and destroys favorable soil microbes (Ma et al., 2023). Soil microbes are key players in nutrient cycling, energy flow, and maintenance of ecosystem stability (Liu et al., 2025; Wu et al., 2024). Nevertheless, continuous cropping decreases the abundance and activity of favorable microbes leading to soil ecological imbalances (Li et al., 2023).

The crop diversification is considered as an important way to increase nutrient cycling, crop production, and microbial activities (Trinchera et al., 2022). The crop diversification affects the soil microorganism owing to differences in soil disturbance, and availability of substrate quality and quantity (Trinchera et al., 2022). For example, increase in cropping diversity results in production of diversified residues which have different chemical composition, therefore, this diversification supports microbial density and diversity. Additionally, different plant species have varied root architectures and they attract particular microbial populations by releasing root exudates and signaling chemicals, which expand the microbial community and may have legacy impact on future crops (van der Bom et al., 2020). Further in diversified cropping systems different straws are returned to field which also have a significant impact on microbial diversity and abundance (Zhang et al., 2020). The middle reaches of the Yangtze River are great grain base commodity in China. Generally, triple cropping and double cropping rice production is used in this region.

However, in recent times people are diverted to single cropping rice due to labor shortage, decline in agricultural benefits and increase in production cost. This change has led to a decline in soil quality and fertility, rice (*Oryza sativa* L.) yield and reduction in the area where double-cropping rice planted (Lei et al., 2022). Therefore, developing rational patterns for paddy-upland multiple cropping rotation in the southern double-cropping rice area is crucial for the efficient use of agricultural resources, soil improvement, and economic and ecological benefits. The changes in planting structure inevitably affect the soil properties which, therefore, affect the diversity and community structure of microbes in paddy field. For instance, Pu et al. (2023) examined the effects of paddy-upland multiple cropping rotation on the soil bacterial community structure. They found that *Ascomycetes* and *Acidobacteria* were dominant bacterial species in different 105 rotations like rapeseed, spring maize, Chinese milk vetch and faba bean in combination with rice. According to Deng et al. (2019) using a no-tillage straw mulching improved the abundance of *Acidobacteria* and *Aspergillus phylum*. According to the research conducted by Xuan et al. (2012), the composition, abundance, and diversity of soil bacterial communities in crop rotation systems are significantly higher than those in rice monoculture. Further, addition of green manures in rotation provides C and N sources to microbes which can improve the bacterial community and diversity in rice field (Deng et al., 2019). However, the above studies mainly focused on the traditional double rice cropping and rotation patterns. There is no study available about the impact of combined winter green manure and paddy-upland multiple cropping rotation mode on soil microbial community structure and diversity and soil properties. Therefore, this study aims to bridge this gap by examining the influence of various paddy-upland multiple cropping rotations on soil microbial community structure and diversity and soil properties. Specifically, the research seeks to clarify how different paddy farming measures affect the composition of soil microbial communities. The ultimate goal was to provide a theoretical and scientific foundation for optimal adjustments to cropping systems and to establish sustainable soil management measures in farmland.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the rice experimental field (28°46′ N, 115°55′ E) of Jiangxi Agricultural University Science and Technology from October 2018 to December 2020. The experimental site belongs to subtropical monsoon humid climate. The study site has average annual total solar radiation of 6330.25 MJ m^{-2}, average temperature of ≥ 10 °C, and annual precipitation of 1921.4 mm. The daily average temperature and precipitation changes during the test period are shown in Figure 1. The tested soil was red clay (corresponding to Ultisols in the USDA Soil Taxonomy and Acrisols in the FAO World Reference Base for Soil Resources (WRB) and it had pH (5.22), organic matter content (28.56 g kg^{-1}), total N content (1.79 g kg^{-1}), available P (27.48 mg kg^{-1}), available K (103.74 mg kg^{-1}), electrical conductivity (CE; 0.3 dS m^{-1}) and cation exchange capacity (CEC; 16 $cmol_{(+)}$ kg^{-1}).

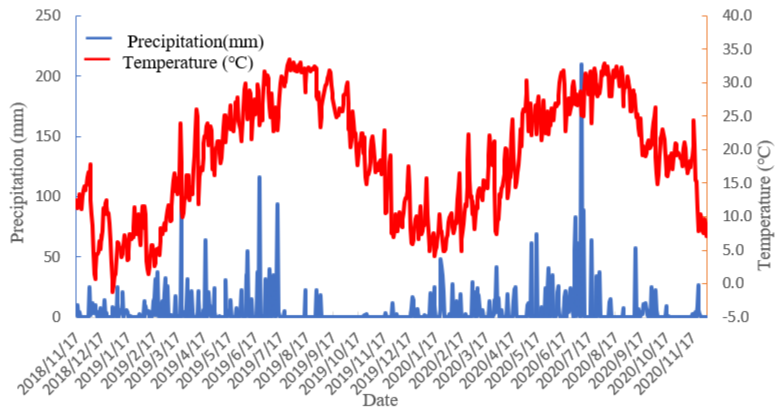

Figure 1. Mean daily temperature and precipitation during the test period.

Experimental design

The study comprised different experimental treatments (Table 1): Chinese milk vetch (*Astragalus sinicus* L.)-double cropping rice (*Oryza sativa* L.) (CRR) as the control (CK), and four planting patterns including rape (*Brassica napus* L.)-early rice-late rice (RRR), potato (*Solanum tuberosum* L.)-early rice-late rice (PRR), Chinese milk vetch-early rice-sweet potato (*Ipomoea batatas* (L.) Lam.)||late soybean (*Glycine max* (L.) Merr.) (CRI), and rape-early rice-sweet potato||late soybean (RRI). Here - indicates continuous planting and || indicates intercropping. Chinese milk vetch and rape were evenly sown and potato slices were soaked, planted. The sowing rate of Chinese milk vetch and rape were 37.5 kg ha^{-1}, 15 kg ha^{-1}, respectively; and the planting density of potato was 73 000 plants ha^{-1}. All winter crops were turned back to the field 15 d before rice transplanting, and the amount of straw returning to the field in winter is shown in Table 2. The field was ploughed twice to create puddled conditions for transplanting of rice. Thereafter, 30 d old seedlings of rice were transplanted in both early and late rice seasons. The seeds of soybean, Chinese milk vetch, rape and soybean were purchased from Jingzhou Xiongfeng seed industry, Hubei Province, China. Sweet potato and late soybean were planted by furrowing and ridging method. The ridge width was 1.2 m and the ridge height was 0.35 m. Each ridge was planted with 4 rows of soybeans, 1 row of sweet potato, 2 rows of soybeans on both sides of sweet potato, with 0.3 m row spacing, 0.25 m plant spacing, 0.2 m row spacing and 0.2 m plant spacing between soybeans. The sowing rate of soybean was 150 kg ha^{-1}. The specific planting time, fertilization amount and fertilization method are shown in Table 3, and other field management was kept constant to get good stand establishment.

Table 1. Details of experimental treatments used in study. Chinese milk vetch double cropping rice (CRR) as the control (CK), and four planting patterns including Chinese milk vetch-early rice-sweet potato||late soybean (CRI), rape early rice-late rice (RRR), rape-early rice-sweet potato||late soybean (RRI), and potato early rice-late rice (PRR). -: Continuous planting; ||: intercropping.

Treatment	Cropping pattern
CRR (CK)	Chinese milk vetch-early rice-late rice
CRI	Chinese milk vetch-early rice-sweet potato||late soybean
RRR	Rape-early rice-late rice
RRI	Rape-early rice-sweet potato||late soybean
PRR	Potato-early rice-late rice

Table 2. Quantity of winter crop straw returned to field during both years of study. Means followed by different letters within a row (or column) are significantly different according to Fisher's least significant difference (LSD) test at $p < 0.05$. Chinese milk vetch double cropping rice (CRR) as the control (CK), and four planting patterns including Chinese milk vetch-early rice-sweet potato||late soybean (CRI), rape early rice-late rice (RRR), rape-early rice-sweet potato||late soybean (RRI), and potato early rice-late rice (PRR). -: Continuous planting; ||: intercropping.

		2019		2020	
Treatments	Crops	Fresh weight	Dry weight	Fresh weight	Dry weight
		kg ha^{-1}		kg ha^{-1}	
CRR (CK)	Chinese milk vetch	31527.9^{b}	6107.53^{ab}	33528.87^{b}	6405.92^{ab}
CRI	Chinese milk vetch	34651.37^{a}	6583.76^{a}	36690.28^{a}	6812.34^{a}
RRR	Rape	20611.89^{d}	5173.58^{c}	23148.61^{c}	5902.90^{c}
RRI	Rape	23169.33^{c}	5757.57^{b}	24327.47^{c}	6348.51^{b}
PRR	Potato	18435.17^{d}	3746.03^{d}	20314.5^{d}	4022.27^{d}

Table 3. Details of field management practices performed during study.

Crop	Variety	Cropping date-Harvest date	Cropping pattern	Fertilizing amount (kg ha^{-1})
Chinese milk vetch	Yujiang big leaf seed	30 Sep 2018-7 Apr 2019 30 Sep 2019-7 Apr 2020	Broadcast sowing	Calcium magnesium phosphate 45
Rape	Deyou 558	8 Nov 2018-7 Apr 2019 6 Nov. 2019-7 Apr 2020	Broadcast sowing	63.75 N, 45 P_2O_5, 225 K_2O
Potato	Dongnong 303	26 Nov 2018-10 Apr 2019 28 Nov 2019-10 Apr 2020	Drill seeding	63.75 N, 45 P_2O_5, 225 K_2O
Soybean	Kuixian II	1 Aug 2019-25 Oct 2019 18 Aug 2020-10 Nov 2020	Hole seeding	150 N, 150 P_2O_5, 375 K_2O
Sweet potato	Guangshu 87	1 Aug 2019-31 Oct 2019 18 Aug 2020-17 Nov 2020	Drill seeding	80 N, 375 P_2O_5, 80 K_2O
Early rice	Zhongjiazao 17	26 Apr 2019-24 Jul 2019 4 May 2020-30 Jul 2020	Transplanting	180 N, 90 P_2O_5, 120 K_2O
Late rice	Tianyou Huazhan	3 Aug 2019-30 Oct 2019 2 Aug 2020-3 Dec 2020	Transplanting	180 N, 90 P_2O_5, 120 K_2O

Determination of soil chemical properties

A five-point sampling method was used to collect soil samples from each plot during the winter crop tilling period, early rice and late rice ripening period. These samples, were taken from the 0-20 cm layer of soil and analyzed to determine different soil properties. The soil pH was measured by pH meter, and soil organic matter was measured using the potassium dichromate method, which is a concentrated sulfuric acid external heating method. Moreover, soil total N (TN) was determined with the Kjeldahl method, and soil available P (AP) and available K (AK) was measured with potassium sodium bicarbonate extraction, ammonium acetate extraction methods. The soil nitrate N (NON) and ammonium N (NHN) were determined by KCl extraction methods. Furthermore, dissolved organic C (DOC), microbial biomass C (MBC), C (AOC), readily oxidized organic C (ROC), and soil organic C (SOC) was determined by following standard procedures (Lefroy et al., 1993; Haynes, 2000; Lu, 2000; Wu, 2006) and details of these procedures are presented in Table 4.

Table 4. Soil chemical property determination project and analytical method.

Measurement indicator	Measurement method
Soil pH	pH meter
Soil organic matter content	Potassium dichromate method-concentrated sulfuric acid external heating
Total N content	Semi-micro Kjeldahl method
Available P content	$NaHCO_3$ leaching-Molybdenum antimony colorimetric method
Available K	NH_4OAc leaching-Flame photometric method
Nitrate N	Ultraviolet spectrophotometric method
Ammonium N	KCl extraction-indophenol blue colorimetric method

Determination of soil bacterial community diversity in paddy fields

The soil samples were collected in late rice season of 2020 to determine the impact of different planting treatments on soil bacterial community diversity. The samples were collected using the five-point sampling method. The samples were taken from 0-20 cm of the cultivated soil with an auger. They were immediately transported to the laboratory and preserved with liquid nitrogen at -80 °C. Then samples were subjected to soil bacterial 16S sequences using the MiSeq (Illumina, San Diego, California, USA) platform high-throughput sequencing method (Meijie Bio-pharmaceutical Science and Technology, Shanghai, China). Further, Amplicon sequencing analysis was conducted with the following main steps.

Genomic DNA extraction and PCR amplification

To extract the genomic DNA from the samples, either CTAB or SDS was used. The purity and concentration of the DNA were then checked through agarose gel electrophoresis. An appropriate amount of the sample was taken into a centrifuge tube and diluted with sterile water to 1 ng μL^{-1}. The diluted genomic DNA was utilized as a template for PCR. Primers with Barcode were selected based on the sequencing region. To ensure amplification efficiency and accuracy, the Phusion High-Fidelity PCR Master Mix with GC buffer and high-fidelity enzyme from New England Biolabs (Ipswich, Massachusetts, USA) were utilized. Phusion High-Fidelity PCR Master Mix with GC buffer and high-efficiency high-fidelity enzymes were used for PCR to guarantee successful amplification and accuracy.

Mixing and purification of PCR products

To detect PCR products, we used electrophoresis with a 2% agarose gel. We mixed equal amounts of samples based on the concentration of the PCR products. After sufficient mixing, we detected the PCR products again using electrophoresis with a 2% agarose gel. We then used the gel recovery kit provided by QIAGEN (Germantown, Maryland, USA) to recover the target bands. The library was constructed using DNA PCR-Free Sample Preparation Kit (TruSeq, Illumina). The constructed library was quantified by Qubit and Q-PCR, and after the library was qualified, it was subjected to on-line sequencing using HiSeq 2500 PE250 (Illumina).

Data analysis

The various treatments of each indicator were processed using Microsoft Excel 2019 (Microsoft Corporation, Redmond, Washington, USA) for data processing. SPSS Statistics 20.0 (IBM Corp., Armonk, New York, USA) was used to compare the significance of soil bacterial diversity (in the late rice season of 2020), as well as the quantity of winter crop straw and soil nutrient content indices (each year separately), through one-way ANOVA. Further mean separation was conducted using Fisher's s least significant difference (LSD, p = 0.05). Additionally, Canoco 5 (Microcomputer Power, Ithaca, New York, USA) was employed for principal coordinate analysis (PCoA) of the structural composition of the bacterial community at the phylum level under various cropping modes. We also conducted redundancy analysis (RDA) of the structural composition of the bacterial community at the genus level, considering the soil chemical properties. Heatmap analyses were conducted to study the correlation between bacterial community composition and soil properties. To do this, we used R software version 4.0.3 (R Core Team, Vienna, Austria) and ran the "pheatmap" package with Spearman correlation coefficients. The clustering was also performed at both the environmental factor level and the species level using the average linkage clustering method. Moreover, OriginPro 8.5 (OriginLab Corporation, Northampton, M assachusetts, USA) was used to generate graphs.

RESULTS

Effects of different planting patterns on soil chemical properties in paddy field

The results regarding the impact of different plant patterns on soil properties are presented in Table 5. The results indicated a substantial impact of different treatments on soil properties. The results indicated treatment RRI had the highest pH in winter cropping in 2019, which was 3.87% higher than the control (Table 5). There was also a significant ($P < 0.05$) difference between treatments in both early and late rice seasons for soil pH. The results also indicated that different treatments also significantly ($P < 0.05$) impacted the soil nutrient availability. In early and late seasons, CRR and RRR had significantly higher N contents than the other treatments. In winter and early rice seasons, nitrate N contents in CRI and PRR treatments significantly increased by 115.58%, 183.12%, 188.46%, and 242.31% than the control respectively (Table 5). On the other hand, in late season, the soil nitrate N in CRI and RRI treatments showed a decrease of 48.35% to 69.23% than the control. In addition, RRI also showed lower ammonium N contents throughout the growing season and maximum reduction of 46.05% was observed in late rice seasons which minimum reduction of 21.11% was observed in winter seasons (Table 5). Different treatments also significantly impacted the soil ammonium N, available P and K. In early season rice, ammonium N in CRI and RRI was decreased by 7.52% and 16.28% than control respectively (Table 5). In winter season, the concentration of available P in CRI, RRI and PRR was increased by 49.63%, 82.98% and 92.16%, respectively. In late rice the available P content in treatment CRI (62.69%) and PRR (70.34%) was significantly higher ($P < 0.05$) than in the control. Moreover, in winter season, PRR and RRR had higher K contents as compared to control. However, PPR substantially increased K concentration, however, maximum increase of 71.80% was seen in early rice, while an increase of 48.26% was seen in late rice. Different treatments also showed a significant impact of soil organic matter contents, however, greatest increase of 20.63% and 20.98% was recorded in RRI with early and late rice seasons.

During second year (2020) different treatments also showed a significantly impact of soil pH and soil nutrient availability. The maximum increase of 4.41% and 4.99% in soil pH was observed with CRI and RRI and early and late planted rice. In 2020, it was observed the CRI treatment increased N contents 15.22% while PRR increased soil N contents by 6.53% respectively (Table 5). The nitrate N contents in PRR and CRI was also increased by 73.42% and 101.27% respectively in winter and early rice while in late season rice nitrate N contents in CRI and RRI was lower than other treatments. In winter cropping season, CRI had maximum ammonium N; however, in early and late rice seasons both CRI and RRI had lower ammonium N as compared to control. The available P contents in treatments CRI, RRI, and PRR were significantly higher ($P < 0.05$) than control at winter and early rice seasons. In the late rice season, treatment CRI showed an increase of 40.32% while treatment PRR showed an increase of 88.19% in soil P concentration. Moreover, the available K content in PRR was significantly higher ($P < 0.05$) than that of the control. In early and late rice seasons of 2020, all the treatments enhanced the organic matter content. Specifically, CRI significantly enhanced the organic matter content by 7.52% and 13.01% in early and late rice seasons ($P < 0.05$).

Table 5. Soil nutrient content of different cropping patterns during 2019 and 2020. Means followed by different letters within a row (or column) are significantly different according to Fisher's least significant difference (LSD) test at $p < 0.05$. Chinese milk vetch double cropping rice (CRR) as the control (CK), and four planting patterns including Chinese milk vetch-early rice-sweet potato||late soybean (CRI), rape early rice-late rice (RRR), rape-early rice-sweet potato||late soybean (RRI), and potato early rice-late rice (PRR). -: Continuous planting; ||: intercropping.

Year/Season	Treatment	pH	Total N	NO_3^--N	NH_4^+-N	Available P	Available K	Organic matter
			g kg^{-1}	mg kg^{-1}	mg kg^{-}	mg kg^{-1}	mg kg^{-1}	g kg^{-1}
2019								
Maturity of winter crops	CRR (CK)	5.17 ± 0.06^{c}	2.13 ± 0.11^{a}	0.77 ± 0.09^{b}	1.80 ± 0.09^{ab}	25.85 ±2.74^{b}	56.02 ± 7.09^{c}	32.55 ± 3.09^{a}
	CRI	5.32 ± 0.01^{ab}	1.89 ± 0.05^{ab}	1.66 ± 0.30^{a}	1.94 ± 0.13^{a}	38.92 ± 3.17^{a}	56.40 ± 5.16^{c}	30.37 ± 1.98^{a}
	RRR	5.21 ± 0.07^{b}	2.10 ± 0.05^{a}	0.50 ± 0.03^{b}	1.67 ± 0.14^{abc}	24.73 ± 1.98^{b}	81.04 ± 5.51^{b}	34.66 ± 1.79^{a}
	RRI	5.37 ± 0.03^{a}	1.72 ± 0.05^{b}	0.83 ± 0.10^{b}	1.42 ± 0.02^{c}	38.68 ± 3.35^{a}	59.28 ± 6.12^{bc}	30.91 ± 1.58^{a}
	PRR	5.11 ± 0.06^{c}	1.98 ± 0.08^{a}	2.18 ± 0.22^{a}	1.59 ± 0.05^{bc}	47.30 ± 2.11^{a}	143.45 ± 11.36^{a}	33.26 ± 0.48^{a}
Maturity of early rice	CRR (CK)	5.30 ± 0.07^{a}	1.93 ± 0.04^{a}	0.26 ± 0.08^{b}	6.12 ± 0.51^{a}	18.25 ± 1.05^{c}	61.14 ± 5.30^{b}	30.54 ± 1.02^{b}
	CRI	5.44 ± 0.10^{a}	1.71 ± 0.05^{b}	0.75 ± 0.11^{a}	5.66 ± 0.38^{a}	26.83 ± 0.38^{b}	50.81 ± 5.58^{b}	34.12 ± 1.92^{a}
	RRR	5.32 ± 0.04^{a}	1.89 ± 0.01^{a}	0.83 ± 0.14^{a}	6.65 ± 0.53^{a}	19.03 ± 0.37^{c}	73.32 ± 9.88^{b}	35.26 ± 0.53^{a}
	RRI	5.45 ± 0.09^{a}	1.56 ± 0.05^{b}	0.14 ± 0.04^{b}	5.81 ± 0.82^{a}	23.13 ± 0.37^{b}	49.70 ± 2.62^{b}	36.84 ± 0.94^{a}
	PRR	5.31 ± 0.07^{a}	1.70 ± 0.06^{b}	0.89 ± 0.12^{a}	5.52 ± 0.44^{a}	35.07 ± 2.34^{a}	105.04 ± 11.20^{a}	28.97 ± 1.34^{b}
Maturity of late rice	CRR (CK)	5.22 ± 0.06^{a}	2.07 ± 0.10^{a}	0.91 ± 0.0.6^{b}	2.15 ± 0.13^{b}	18.44 ± 2.44^{b}	57.33 ± 2.33^{b}	28.88 ± 0.80^{b}
	CRI	5.33 ± 0.09^{a}	1.79 ± 0.05^{b}	0.47 ± 0.13^{c}	1.80 ± 0.04^{b}	30.00 ± 2.65^{a}	61.33 ± 2.60^{b}	32.80 ± 1.04^{a}
	RRR	5.22 ± 0.02^{a}	1.89 ± 0.01^{a}	0.87 ± 0.09^{b}	3.30 ± 0.07^{a}	16.10 ± 1.12^{b}	69.67 ± 5.70^{b}	32.36 ± 0.79^{a}
	RRI	5.35 ± 0.03^{a}	1.56 ± 0.05^{b}	0.28 ± 0.03^{c}	1.16 ± 0.14^{c}	24.73 ± 4.91^{ab}	64.67 ± 2.33^{b}	34.94 ± 0.30^{a}
	PRR	5.23 ± 0.06^{a}	1.70 ± 0.06^{b}	1.91 ± 0.10^{a}	2.93 ± 0.18^{a}	31.41 ± 3.96^{a}	85.00 ± 7.51^{a}	28.43 ± 0.86^{b}
2020								
Maturity of winter crops	CRR (CK)	5.08 ± 0.07^{a}	2.11 ± 0.8^{a}	0.79 ± 0.02^{c}	0.59 ± 0.13^{b}	20.18 ± 1.42^{b}	66.33 ± 3.53^{bc}	33.89 ± 0.37^{a}
	CRI	5.21 ± 0.06^{a}	1.94 ± 0.06^{b}	1.59 ± 0.15^{a}	1.47 ± 0.21^{a}	37.14 ± 2.67^{a}	60.00 ± 2.52^{c}	31.36 ± 0.57^{b}
	RRR	5.13 ± 0.03^{a}	1.97 ± 0.03^{ab}	0.86 ± 0.16^{c}	0.41 ± 0.07^{b}	22.86 ± 2.57^{b}	77.33 ± 5.36^{b}	31.35 ± 0.61^{b}
	RRI	5.26 ± 0.05^{a}	1.83 ± 0.03^{b}	1.15 ± 0.13^{bc}	0.41 ± 0.10^{b}	33.14 ± 2.32^{a}	59.33 ± 2.33^{c}	33.38 ± 0.71^{a}
	PRR	5.11 ± 0.03^{a}	1.88 ± 0.03^{b}	1.37 ± 0.11^{ab}	0.50 ± 0.22^{b}	40.35 ± 3.78^{a}	93.67 ± 2.85^{a}	33.03 ± 0.43^{a}
Maturity of early rice	CRR (CK)	5.21 ± 0.07^{c}	1.96 ± 0.04^{ab}	0.08 ± 0.01^{c}	5.58 ± 2.19^{a}	18.60 ± 1.06^{b}	19.67 ± 1.86^{bc}	31.38 ± 0.36^{b}
	CRI	5.44 ± 0.07^{ab}	2.04 ± 0.04^{a}	0.49 ± 0.02^{a}	4.81 ± 1.67^{a}	26.81 ± 1.92^{a}	14.33 ± 0.33^{c}	33.74 ± 1.34^{a}
	RRR	5.27 ± 0.04^{bc}	1.88 ± 0.04^{b}	0.21 ± 0.06^{b}	3.85 ± 1.39^{a}	16.32 ± 0.95^{b}	22.33 ± 2.03^{b}	31.72 ± 0.97^{ab}
	RRI	5.47 ± 0.04^{a}	1.94 ± 0.05^{ab}	0.17 ± 0.03^{bc}	3.50 ± 0.96^{a}	29.59 ± 2.75^{a}	16.00 ± 1.15^{c}	32.49 ± 0.37^{ab}
	PRR	5.32 ± 0.06^{abc}	1.72 ± 0.04^{c}	0.52 ± 0.05^{a}	2.24 ± 1.28^{a}	29.90 ± 2.50^{a}	38.33 ± 2.19^{a}	32.30 ± 0.73^{b}
Maturity of late rice	CRR (CK)	5.24 ± 0.04^{a}	1.99 ± 0.07^{ab}	2.03 ± 0.25^{a}	7.02 ± 0.36^{a}	22.10 ± 1.94^{c}	13.67 ± 1.45^{c}	33.14 ± 0.36^{b}
	CRI	5.30 ± 0.07^{a}	2.12 ± 0.10^{a}	1.66 ± 0.15^{a}	6.59 ± 0.24^{a}	31.01 ± 0.83^{b}	19.33 ± 2.03^{abc}	37.45 ± 0.18^{a}
	RRR	5.25 ± 0.04^{a}	1.98 ± 0.03^{ab}	2.78 ± 0.47^{a}	7.18 ± 0.26^{a}	22.55 ± 2.43^{c}	20.67 ± 1.45^{ab}	33.78 ± 0.99^{ab}
	RRI	5.34 ± 0.04^{a}	2.00 ± 0.04^{ab}	1.79 ± 0.59^{a}	6.68 ± 0.48^{a}	27.72 ± 2.21^{bc}	17.33 ± 2.40^{bc}	35.57 ± 1.11^{ab}
	PRR	5.27 ± 0.03^{a}	1.84 ± 0.06^{b}	2.35 ± 0.40^{a}	6.47 ± 0.25^{a}	41.59 ± 3.73^{a}	24.33 ± 1.33^{a}	33.92 ± 0.71^{ab}

Effects of different planting patterns on soil bacterial community structure and diversity in paddy field

The soil samples were collected in late rice season in 2020 to extract DNA. Then DNA sequence was performed and in total we obtained 929 725 high-quality sequences from 15 soil samples. The average length of the sequences was 415 base pairs, and the coverage index of each sample was above 98%, indicating sequenced bacterial 16S rDNA had great depth. The bacterial community abundance and diversity indices differed across different cropping patterns (Table 6). The diversity indices of soil across various cropping patterns did not show any significant differences. The results indicated that RRR, CRR, and RRI treatments proved beneficial in improving Shannon, sobs, Chao1, and abundance-based coverage estimator (ACE) indices. However, RRR treatment proved more effective in improving soil diversity indices indicating that planting rapeseed and Chinese milk vetch in winter is beneficial to increase bacterial abundance and diversity.

Proteobacteria (16.86% to 21.70%), *Chloroflexi* (16.25% to 21.02%), and *Actinobacteria* (13.49% to 20.75%) were recognized as most dominant bacterial groups (Figure 2). Next were *Acidobacteria* (11.40% to 17.03%), *Firmicutes* (5.62% to 9.17%), *Myxococcota* (3.05% to 3.42%), *Bacteroidota* (2.27% to 3.81%), *Desulfobacterium*

Desulfobacterota (2.07% to 3.22%), *Nitrospirae* (1.66% to 2.44%), and *Gemmatimonadota* (1.64% to 2.40%) (Figure 2). Further, RRR and PRR treatments increased *Chloroflexi* abundance by 29.35% and 28.62% respectively than CRI ($P < 0.05$). Conversely, the *Proteobacteria* was more abundant in treatments CRI and CRR and in both treatments *Proteobacteria* abundance was increased by 28.71% and 11.33 respectively than RRR. Further, CRI and RRI increased the relative abundance of *Actinobacteria* and *Bacillus phyla* by 53.82% and 29.73% respectively than control. The relative abundance of *Firmicutes* CRI (63.17%), RRI (32.38%), and PRR was increased by 63.17%, 32.38% and 28.47% respectively than the control. The relative abundance of *Acidobacteria* in all treatments was significantly higher ($P < 0.05$) than that of the control, with increase ranged from 32.98 to 49.39% respectively.

Table 6. Alpha diversity index of bacterial operational taxonomic unit (OTU) under different cropping patterns. ACE: Abundance-based coverage estimator. Means followed by different letters within a row (or column) are significantly different according to Fisher's least significant difference (LSD) test at $p < 0.05$. Chinese milk vetch double cropping rice (CRR) as the control (CK), and four planting patterns including Chinese milk vetch-early rice-sweet potato||late soybean (CRI), rape early rice-late rice (RRR), rape-early rice-sweet potato||late soybean (RRI), and potato early rice-late rice (PRR). -: Continuous planting; ||: intercropping.

	Alpha diversity index of bacterial OTU			
Treatment	Shannon	sobs	Chao1	ACE
CRR (CK)	7.03^a	3903^a	4986.45^a	5054.94^a
CRI	6.84^a	3655^a	4742.88^a	4759.22^a
RRR	7.04^a	3951^a	5069.15^a	5132.27^a
RRI	6.99^a	3819^a	4897.54^a	4940.93^a
PRR	6.96^a	3745^a	4872.89^a	4880.74^a

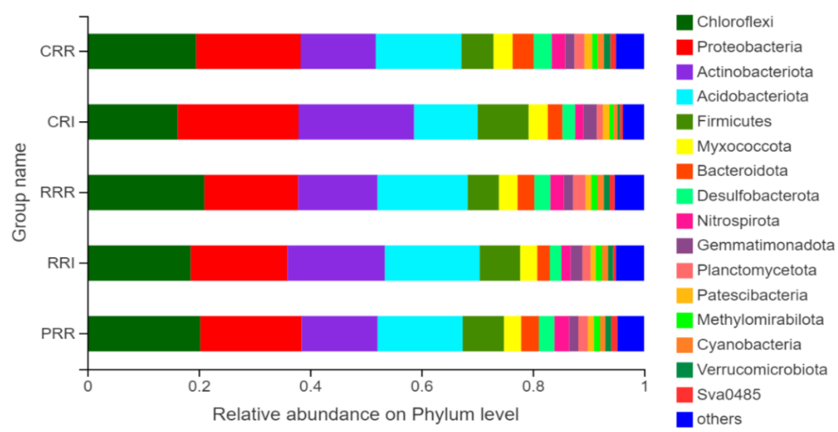

Figure 2. Relative abundances of soil bacteria at the phylum level of different cropping patterns. Chinese milk vetch double cropping rice (CRR) as the control (CK), and four planting patterns including rape early rice-late rice, potato early rice-late rice (PRR), Chinese milk vetch-early rice-sweet potato||late soybean (CRI), and rape-early rice-sweet potato||late soybean (RRI). -: Continuous planting; ||: intercropping.

The PCoA analyses indicated significant differences in the soil bacterial community structure under different cropping patterns (Figure 3). The PC1 account for 62.99% of the alterations in the bacterial community, while the PC2 axis showed 12.69% of the changes in the bacterial community. The soil bacterial community structure of treatments CRR, RRR, and PRR were closely distributed in the projection of the PC1 axis, indicating similar bacterial community structure (Figure 3). Nevertheless, each treatment possesses its distinct bacterial community. This suggests that winter planting of various crops is favorable for diversifying the soil bacterial community structure. At PC2 axis, the bacterial community structure of CRI and RRI treatments was observed further away from the distribution of other treatments. This indicates that the bacteria community structure in CRI and RRI treatments differed more than CRR, RRR, and PRR treatments. Regarding internal variability, the soil bacteria community structure had more significant variability in CRI and RRI treatments and less in CRR, RRR, and PRR treatments. This suggests that the heterogeneity within the sample sites in the paddy-upland cropping treatment was higher than in the double-cropping rice treatment.

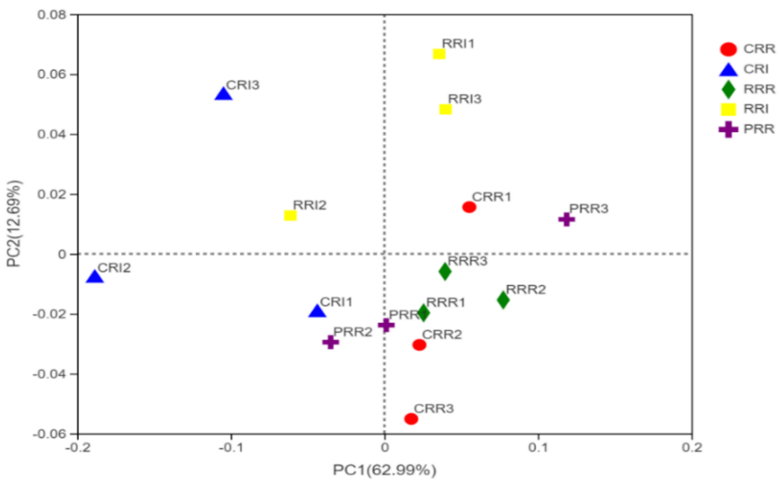

Figure 3. Principal coordinate analysis (PCoA) analysis of soil bacteria community structure of different cropping patterns. Chinese milk vetch double cropping rice (CRR) as the control (CK), and four planting patterns including rape early rice-late rice, potato early rice-late rice (PRR), Chinese milk vetch-early rice-sweet potato||late soybean (CRI), and rape-early rice-sweet potato||late soybean (RRI). -: Continuous planting; ||: intercropping.

Relationship between soil properties and soil bacterial communities

Redundancy analysis (RDA) was used to analyze soil bacterial community structures in different cropping patterns in paddy fields (Figure 4). The results showed that the first axis explained 29.02% variation and second axis showed a variation of 9.53%. The bacterial communities showed a significant correlation with DOC, MBC, and AOC. Further, NHN, and AK were more closely linked to the CRR, RRR, and PRR treatments, while DOC, MBC, ROC, AOC, SOC, TN, AP, and pH were more closely associated with the CRI and RRI treatments. The correlation between the bacterial community structure and soil properties was also studied by analyzing heat maps (Figure 5). The analysis was done at the level of the top 30 species of relative abundance in paddy soils. The results showed DOC was positively correlated with the relative abundance of *Actinobacteria*, *Bacilli Gemmatimonadetes* and *Thermoleophilia*. However, it was negatively correlated with *Planctomycetes, Thermodesulfovibrionia, Anaerolineae, Kryptonia*, and *Vicinamibacteria*. There was a positive between MBC *Bacteriophage*, *Thermolithobacterium*, and *Bacilli*, but a negative correlation between MBC and *Anaerolineae, Kryptonia*, and *norank__p__Sva0485*. On the other hand, SOC showed a significant positive correlation with *Bacilli*, *Gemmatimonadetes*, and *Bacteroidia* while a negative correlation with *Thermodesulfovibrionia, Bacteroidia, Kryptonia* and *norank__p__Sva0485*. There was also a positive correlation between nitrate N and of *Thermodesulfovibrionia*, *Bacteroidia*, *Myxococcia*, *Anaerolineae*, *Aminicenantia*, and *Subgroup_18*, *Kryptonia*. On the other hand, there was a significant negative correlation between nitrate N and *Bacilli*. Similarly, there was a significant positive correlation between the ammonium N and *Thermodesulfovibrionia, Bacteroidia*, and *4-29-1* and a negative correlation between nitrate N and *Bacilli*. Lastly, soil pH, AK, and TN contents did not significantly affect the changes in the relative abundance of bacteria.

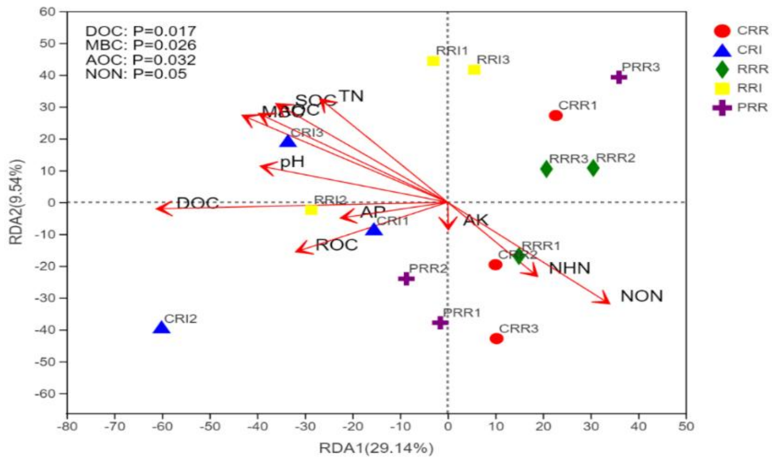

Figure 4. Redundancy analysis (RDA) analysis between soil bacterial community structure and soil chemical properties of different cropping patterns. Chinese milk vetch double cropping rice (CRR) as the control (CK), and four planting patterns including rape early rice-late rice, potato early rice-late rice (PRR), Chinese milk vetch-early rice-sweet potato||late soybean (CRI), and rape-early rice-sweet potato||late soybean (RRI). -: Continuous planting; ||: intercropping. ROC: Readily oxidized organic C; DOC: dissolved organic C; AP: available P; MBC: microbial biomass C; TN: total N; SOC: soil organic C; AOC: active organic C; AK: available K; NHN: ammonium N; NON: nitrate N.

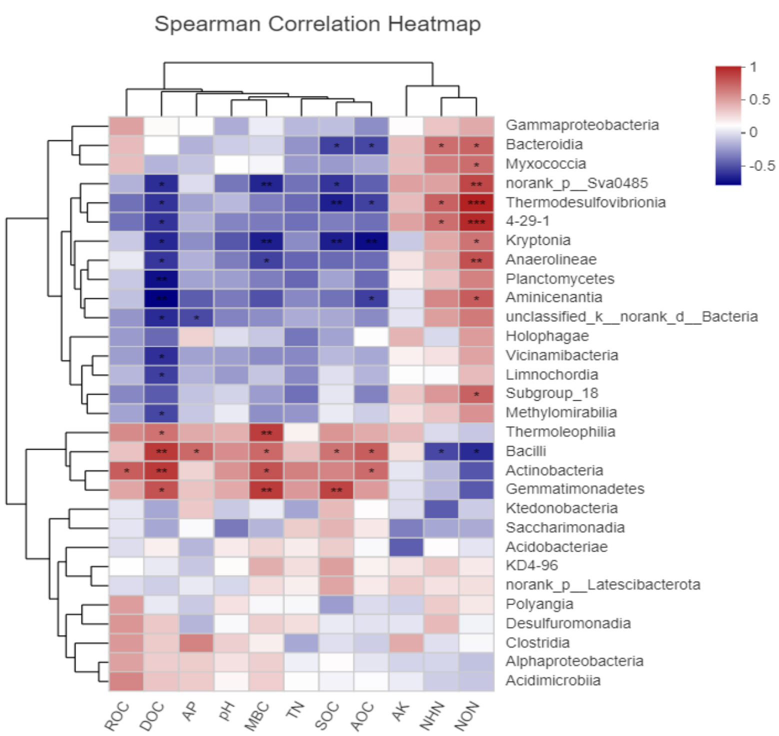

Figure 5. Heatmap between soil bacterial community compositions in class level and soil chemical properties of different cropping patterns. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. ROC: Readily oxidized organic C; DOC: dissolved organic C; AP: available P; MBC: microbial biomass C; TN: total N; SOC: soil organic C; AOC: active organic C; AK: available K; NHN: ammonium N; NON: nitrate N.

DISCUSSION

Effects of different cropping patterns on chemical properties in paddy fields

The results indicated that different treatments significantly improved the soil nutrient concentration and soil organic matter. The return of different winter crops and multiple cropping effectively enhanced soil nutrient availability and organic matter. Straw contains essential nutrients and when it decomposes in soil it releases different nutrients, adds organic matter, and increases microbial activity which enhances the decomposition of organic matter (OM). Besides this, straw returning also improves soil structure and all these changes lead to an increase in soil nutrient availability (Yang et al., 2024). Multiple cropping also improves soil permeability, soil pore structure, and soil organic matter, thereby leading to substantial improvement in soil fertility (Jiang and Zhou, 2019; Liu et al., 2025). Different multiple cropping patterns increased the soil organic C (SOC) concentration which could be an important reason for substantial increase in soil nutrient availability (Arif et al., 2021).

The results of this experiment showed CRI and RRI after 2 yr was conducive to the increase of total N content. This could be attributed to strong N fixation capacity of Chinese milk vetch. Another reason is that planting dry crops (sweet potato, soybean) and other N-fixing crops in late rice season reduces the risk of N loss. In this experiment, different planting patterns had different effects on the content of available P and available K in soil. The content of available P in soil treated with CRI, RRI and PRR was significantly higher than that in control, and the potato-early rice-late rice and rape-early rice-late rice modes were more conducive to the accumulation of available K content in soil. This may be because soil colloids in flooded paddy fields are more likely to absorb K than P which led to a significant increase in soil K and P concentration in these treatments (Zhong et al., 2019). The results indicate CRI enhanced soil P and K availability which is attributed to an increase in soil microbial activity. In addition, the decaying of milk vetch roots also added nutrients into soil including P and K which could also be reasons for a substantial increase in the availability of both these nutrients in CRI treatments (Wang et al., 2023; Fang et al., 2025).

The results of this study showed that compared with the control group, the nitrate N content in soil treated with CRI and RRI was significantly reduced. Both CRI and RRI reduced the residual nitrate N content in soil after crop harvesting, thereby reducing the risk of soil residual N loss. In late rice season, N-fixing crops such as sweet potato and soybean were planted with CRI and RRI treatment, which increased soil microbial activity, and reduced field loss of N fertilizer. It may also be because the return of green fertilizer crops in winter increased the yield of subsequent crops and increased the amount of N carried out by crops which led to reduction in availability of nitrate N.

In this study, pH values of treatment CRI and RRI remained at a high level and this is consistent with the research results of Du et al. (2013), which showed that irrigation drought rotation could improve soil pH and reduce soil acidification. The pH value of early water treatment and late drought treatment (CRI and RRI) remained at a higher level, which weakened soil acidification and was conducive to the increase of total N and organic matter content. The results indicated that different plant patterns also significantly enhanced soil organic matter; however, CRI remained the top performer across both seasons. Chinese milk vetch being a legume fixes N and promotes microbial activities which aids in the decomposition of organic matter (Ma et al., 2020). In addition, milk vetch used in the study contained significant amount of C which also contributed to a substantial increase in soil matter.

Effects of different cropping patterns on soil bacterial community diversity in paddy fields

Soil microorganisms play a vital role in the decomposition organic matter, soil fertility and nutrient availability (Chen et al., 2024). Different cropping patterns implemented in paddy fields did not significantly impact the diversity and richness of soil bacteria. However, the Shannon, sobs, Chao1, and ACE indices of treatments RRR, CRR, and RRI were high. Among these treatments, RRR had the best outcome, suggesting that planting rapeseed and Chinese milk vetch during winter promoted bacterial abundance and diversity owing to addition of organic matter and nutrients (Pu et al., 2023). The green manure crops like milk vetch and rapeseed increased the soil organic matter which affected the soil bacterial community. Soil organic matter provides nutrients and energy to soil microbes, creates a favorable habitat for microbes, and protects the microbes from environmental stress. This in turn increases the abundance and diversity of soil microbes (Chen et al., 2022). Therefore, green crops significantly increased organic matter which contributed to an increase in soil bacterial abundance and diversity. Soil microorganism distribution pattern is affected by soil properties and cropping systems (Fierer and Jackson, 2006). The bacterial community structure in treatments CRR, RRR, and PRR was similar, while CRI and RRI had a more varied and distant bacterial community structure. This suggests that the bacterial communities in CRI and RRI treatments differ from those in CRR, RRR, and PRR, which may be due to differences in soil properties and cropping systems.

The dominant species of bacteria in the soil across different cropping patterns were similar at the phylum level; however, the relative abundance of these species varied across planting patterns. During this experiment, the *Proteobacteria* relative abundance was higher in treatment CRI than in double-cropping rice (CRR, RRR, PRR) treatments. The CRI treatment enhances the soil organic matter and being a legume crop it also provides a nutrient-rich rice environment of bacterial growth. The exudates released from its roots also promote microbial growth by providing organic compounds. Therefore, all these changes led to a significant increase in the abundance of *Proteobacteria* in this treatment. In addition, treatments CRI and RRI increased the relative abundance of the *Bacillus* possibly due to its ability to grow in dry and organic matter-rich environments (Chen, 2019). Consequently, treatments CRI and RRI had a higher relative abundance of *Bacillus* as they were planted with dry crops during the late rice season when the soil was more parched and had higher organic matter content.

Correlation between soil properties and bacterial community structure under different cropping patterns in paddy fields

The community structure of bacteria in the early and late upland cropping treatments (CRI, RRI) was primarily influenced by dissolved organic C (DOC), readily oxidized organic C (ROC), microbial biomass C (MBC), active organic C (AOC), soil organic C (SOC) total N (TN),and pH contents. Opposite to this, the community structure of soil bacteria in the double-cropping rice treatments (CRR, RRR, PRR) was mainly affected by ammonium N (NHN), nitrate N (NON), and available K (AK) contents. Soil microorganisms require the soluble organic matter to sustain their growth and metabolic processes. Specifically, the more active organic C fractions like DOC and MBC, are readily absorbed and utilized by microorganisms. The CRI and RRI had significantly higher levels of DOC and MBC in the experiment than the other treatments which results in better bacterial community structure. Soil pH is the most reliable indicator of the structural composition of bacterial communities (Fierer, 2017). In this experiment, the pH levels of CRI and RRI were higher than other treatments, which significantly impacted the distribution of community structure different treatments.

The contents of DOC and MBC were significantly positively correlated with the relative abundance of *Actinobacteria, Gemmatimonadetes*, and *Thermoleophilia* but significantly negatively correlated with the relative abundance of *Anaerolineae*. There was a positive correlation between the abundance of *Thermodesulfovibrionia*, *Bacteroidia*, *Myxococcia*, and *Anaerolineae* and the content of NON. This suggests that DOC, MBC, and NON was significantly correlated with the abundance of *Anaerolineae*. During the experiment, it was observed that when nitrate N content increased and DOC and MBC content decreased. Then there was an increase in the relative abundance of *Anaerolineae* under *Chloroflexi*. However, in CRI and RRI, where DOC and MBC content increased, the relative abundance of *Anaerolineae* under *Chloroflexi* decreased. This could be because *Chloroflexus* belongs to *Oligotrophic* bacteria, which are inhibited in environments with high nutrient content. Therefore, it can be concluded that treatments CRI and RRI are more favorable to soil C sequestration. Ramirez et al. (2012) discovered that changes in soil MBC content caused by N fertilizer treatments increased the relative abundance of *Actinomyces* and *Firmicutes*. At the same time, there was a decrease in the relative abundance of *Acidophilus*. *Actinobacteria* and *Eutrophic* bacteria can use C sources for fast growth (Chen, 2019) and they can also effectively break down and use lignin and cellulose (Pankratov et al., 2011). In this experiment, the increase of DOC and MBC contents in soils treated with paddy-upland cropping might have promoted the growth of *Actinobacteria*. Additionally, the return of straw from planting dry crops (sweet potato) provided more lignin and cellulose, which further boosted the growth of *Actinomycetes*.

CONCLUSIONS

Planting rapeseed and Chinese milk vetch during winter boosted diversity and community structure of soil bacterial communities. The most common bacterial species in terms of relative abundance were *Chloroflexi*, *Proteobacteria*, and *Actinomycetes*, regardless of the planting pattern. The bacterial community structures of Chinese milk vetch double cropping rice (CRR) rape-early rice-late rice (RRR), potato early rice-late rice (PRR), Chinese milk vetch-early rice-sweet potato//late soybean (CRI), and rape -were significantly different. The soil bacteria community structure in CRI and RRI treatments was primarily influenced by organic C and its fractions, total N, effective P, and pH content. The CRI and RRI treatments particularly increased the total N content and reduced the soil nitrate N, thereby reducing the risk of N loss. Furthermore, these treatments also raised the soil pH, decreasing soil acidification. In contrast, the community structure of soil bacteria in CRR, RRR, and PRR treatments was mainly influenced by available K, nitrate N, and ammonium N content. This study only explored the relationship between soil C pool and bacterial community structure at overall soil level. However, in future studies may be considered from different tilling layers or aggregate levels. Additionally, this study did not explore the functional aspect of microbial communities and their direct impact on crop growth and soil health. Therefore, future studies should aim to examine the impacts of these microbial communities on soil health and crop productivity.

Author contribution

Conceptualization: B.Y., G.H. Formal analysis: Y.H., J.Y., Q.H. Data curation: Y.H., J.Y., Q.H. Writing-original draft: B.Y., G.H. Writing-review & editing: M.U.H., T.A.Y.A., M.H. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgement

This work was supported and funded by National Natural Science Foundation of China (32160528), the National Key R&D Program of China (2016YFD0300208); Modern agricultural industry system in Jiangxi Province-Paddy comprehensive planting and breeding 4industry technology system (JXARS-12).

The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number RGP 2/325/46.

References

Arif, M., Ikramullah, Jan, T., Riaz, M., Akhtar, K., Ali, S., et al. 2021. Biochar and leguminous cover crops as an alternative to summer fallowing for soil organic carbon and nutrient management in the wheat-maize-wheat cropping system under semiarid climate. Journal of Soils and Sediments 21:1395-1407. doi:10.1007/s11368-020-02866-y.

Bedolla-Rivera, H.I., Negrete-Rodríguez, M.D.L.L.X., Gámez-Vázquez, F.P., Álvarez-Bernal, D., Conde-Barajas, E. 2023. Analyzing the impact of intensive agriculture on soil quality: A systematic review and global meta-analysis of quality indexes. Agronomy 13:2166. doi:10.3390/agronomy13082166.

Bargali, S.S., Padalia, K., Bargali, K. 2019. Effects of tree fostering on soil health and microbial biomass under different land use systems in the Central Himalayas. Land Degradation & Development 30:1984-1998. doi:10.1002/ldr.3394.

Chen, J. 2019. Effect of long-term fertilization on soil carbon fractions and microbial characteristics in a rice-wheat rotation system Journal of Plant Nutrition and Fertilizers 25(1):36-44.

Chen, J., Du, Y., Zhu, W., Pang, X., Wang, Z. 2022. Effects of organic materials on soil bacterial community structure in long-term continuous cropping of tomato in greenhouse. Open Life Science 17:381-392. doi:10.1515/biol-2022-0048.

Chen, Q., Song, Y., An, Y., Lu, Y., Zhong, G. 2024. Soil microorganisms: Their role in enhancing crop nutrition and health. Diversity 16:734. doi:10.3390/d16120734.

Deng, C.C., Li, L.L., Xie, J.H., Peng, Z.K., Wang, J.B., Xie, J.H, et al. 2019. Effects of tillage on soil bacterial community in the dryland farming area of central Gansu. Acta Pedologica Sinica 56:207-216.

Du, X.D.X., Luo, L.L., Chen, C.C., Li, S.L., Liu, L.G., Wang, W.F., et al. 2013. Effects of long-term no-tillage direct seeding mode on crop yield and soil physiochemical properties in rice/rapeseed rotation system. Rice Science 21:210-216.

Fang, Y., Li, Q., He, C., Wang, K., Wang, F. 2025. Effects of crop rotation and straw return on soil microbiome in a southern paddy field. Chilean Journal of Agricultural Research 85:57-76. doi:10.4067/S0718-58392025000100057.

Fierer, N. 2017. Embracing the unknown: Disentangling the complexities of the soil microbiome. Nature Reviews of Microbiology 15:579-590. doi:10.1038/nrmicro.2017.87.

Fierer, N., Jackson, R.B. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of National Academy of Sciences of the United States of America 103:626-631. doi:10.1073/pnas.0507535103.

Haynes, R.J. 2000. Labile organic matter as an indicator of organic matter quality in arable and pastoral soils in New Zealand. Soil Biology and Biochemistry 32:211-219. doi:10.1016/S0038-0717(99)00148-0.

Jiang, Y., Zhou, X. 2019. Effects of long-term wheat straw returned to field on crop yield and soil chemical properties in soybean-wheat rotation areas. Transactions of the Chinese Society of Agricultural Engineering 35(24):113-120. doi:10.11975/j.issn.1002-6819.2019.24.014.

Lefroy, R.D., Blair, G.J., Strong, W.M. 1993. Changes in soil organic matter with cropping as measured by organic carbon fractions and 13 C natural isotope abundance. Plant Soil 155:399-402.

Lei, B., Wang, J., Yao, H. 2022. Ecological and environmental benefits of planting green manure in paddy fields. Agriculture 12:223. doi:10.3390/agriculture12020223.

Li, J., Cheng, X., Chu, G., Hu, B., Tao, R. 2023. Continuous cropping of cut chrysanthemum reduces rhizospheric soil bacterial community diversity and co-occurrence network complexity. Applied Soil Ecology 185:104801. doi:10.1016/j.apsoil.2022.104801.

Liu, P., Huang, Z., Tan, S., Wu, W., Chen, T., Qin, J., et al. 2025. Investigating the impact of soil properties on the nutrient content and microbial community of sisal (*Agave sisalana* Perrine). Chilean Journal of Agricultural Research 85:215-231. doi:10.4067/S0718-58392025000200215.

Lu, R.K. 2000. The analysis methods of soils agro-chemistry. China Agricultural Science and Technology Press, Beijing, China.

Ma, Z., Guan, Z., Liu, Q., Hu, Y., Liu, L., Wang, B., et al. 2023. Obstacles in continuous cropping: Mechanisms and control measures. Advances in Agronomy 179:205-256. doi:10.1016/bs.agron.2023.01.004.

Ma, Q., Li, J., Aamer, M., Huang, G. 2020. Effect of Chinese milk vetch (*Astragalus sinicus* L.) and rice straw incorporated in paddy soil on greenhouse gas emission and soil properties. Agronomy 10:717. doi:10.3390/agronomy10050717.

Padalia, K., Bargali, S.S., Bargali, K., Khulbe, K. 2018. Microbial biomass carbon and nitrogen in relation to cropping systems in Central Himalaya, India. Current Science 115:1741-1750.

Pankratov, T.A., Ivanova, A.O., Dedysh, S.N., Liesack, W. 2011. Bacterial populations and environmental factors controlling cellulose degradation in an acidic Sphagnum peat. Environmental Microbiology 13:1800-1814.

Pu, J., Li, Z., Tang, H., Zhou, G., Wei, C., Dong, W., et al. 2023. Response of soil microbial communities and rice yield to nitrogen reduction with green manure application in karst paddy areas. Frontiers in Microbiology 13:1070876. doi:10.3389/fmicb.2022.1070876.

Ramirez, K.S., Craine, J.M., Fierer, N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology 18:1918-27. doi:10.1111/j.1365-2486.2012.02639.x.

Trinchera, A., Migliore, M., Raffa, D.W., Ommeslag, S., Debode, J., Shanmugam, S., et al. 2022. Can multi-cropping affect soil microbial stoichiometry and functional diversity, decreasing potential soil-borne pathogens? A study on European organic vegetable cropping systems. Frontiers in Plant Sciences 13:952910. doi:10.3389/fpls.2022.952910.

van der Bom, F.J., Williams, A., Bell, M.J. 2020. Root architecture for improved resource capture: trade-offs in complex environments. Journal of Experimental Botany 71:5752-5763. doi:10.1093/jxb/eraa324.

Wang, H., Tang, S., Han, S., Li, M., Cheng, W., Bu, R., et al. 2023. Effects of long-term substitution of chemical fertilizer with Chinese milk vetch on soil phosphorus availability and leaching risk in the double rice systems of Eastern China. Field Crops Research 302:109047. doi:10.1016/j.fcr.2023.109047.

Wu, J.S. 2006. Methods for the determination of soil microbial biomass and their application. Meteorological Press, Beijing, China.

Wu, H., Cui, H., Fu, C., Li, R., Qi, F., Liu, Z., et al. 2024. Unveiling the crucial role of soil microorganisms in carbon cycling: A review. Science of the Total Environment 909:168627. doi:10.1016/j.scitotenv.2023.168627.

Xu, M., Lu, X., Xu, Y., Zhong, Z., Zhang, W., Ren, C., et al. 2020. Dynamics of bacterial community in litter and soil along a chronosequence of *Robinia pseudoacacia* plantations. Science of the Total Environment 703:135613. doi:10.1016/j.scitotenv.2019.135613.

Xu, Y., Wang, T., Li, H., Ren, C., Chen, J., Yang, G., et al. 2019. Variations of soil nitrogen-fixing microorganism communities and nitrogen fractions in a *Robinia pseudoacacia chronosequence* on the Loess Plateau of China. Catena 174:316-323. doi:10.1016/j.catena.2018.11.009.

Xuan, D.T., Guong, V.T., Rosling, A., Sadhna, A., Chai, B., Nils, H. 2012. Different crop rotation systems as drivers of change in soil bacterial community structure and yield of rice, *Oryza sativa*. Biology & Fertility of Soils 48(2):217-225. doi:10.1007/s00374-011-0618-5.

Yang, C., Wang, X., Li, J., Zhang, G., Shu, H., Hu, W., et al. 2024. Straw return increases crop production by improving soil organic carbon sequestration and soil aggregation in a long-term wheat-cotton cropping system. Journal of Integrative Agriculture 23:669-679. doi:10.1016/j.jia.2023.06.009

Zhang, S., Wang, Y., Sun, L., Qiu, C., Ding, Y., Gu, H., et al. 2020. Organic mulching positively regulates the soil microbial communities and ecosystem functions in tea plantation. BMC Microbiology 20(1):103. doi:10.1186/s12866-020-01794-8.

Zhang, W., Wei, H., Gao, H., Hu, Y. 2005. Advances of studies on soil microbial diversity and environmental impact factors. Chinese Journal of Ecology 10:48-54.

Zhong, C., Yang, B.J., Zhang, P., Li, P., Huang, G. 2019. Effects of winter crop-based rice-upland rotation patterns on rice yield and CH_4 and N_2O emissions from paddy fields. Journal of Nuclear Agricultural Sciences 33:379-388.