Research article

Winter crop rotation intensification to increase rice yield, soil carbon, and microbial diversity

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ARTICLE INFO

ABSTRACT

Crop rotation has widely contributed to increasing farmland biodiversity as well as to improving soil carbon pools and microbial diversity. However, there is a weak understanding of the suitability of winter crop rotation intensification in double rice fields, especially rotation with various winter crops. For this task, a long-term field experiment based on one from 2012 was conducted with five winter crop systems for double rice: winter fallow (T0), winter milk vetch (T1), winter rape (T2), winter garlic (T3), winter rotation intensification with potato, milk vetch, and rape (T4). Parameters such as crop yield, soil carbon, nitrogen, and soil microorganism were measured. It was found that compared to winter fallow, winter milk vetch, rape, garlic, and crop rotation intensification practices increased the late rice yield by 2.5%, 2.3%, 4.5%, and 3.7%, respectively; winter garlic and crop rotation intensification also increased the early rice yield by 4.6% and 3.5%, respectively. This is associated with the promotion of rice tillering. At the same time, for winter crop rotation, compared to winter fallow, the soil organic carbon increased by 21%. With the input of diversified crop residues, winter crops were effective in soil carbon sequestration, improving soil microbial structure, and increasing soil microbial diversity. The Shannon diversity index of winter crops ranged from 9.75 to 9.91, while winter fallow was 9.38. The Simpson's diversity index of winter crops ranged from 0.997 to 0.998, while winter fallow was 0.996. In conclusion, winter crop practices, especially winter crop rotation intensification, can enhance soil health and sustainability in double rice fields through its positive feedback on crop yield, soil carbon sequestration, and microorganisms.

1. Introduction

During the last few decades, to maximize crop yields and economic benefits, the winter fallow-double rice system has become the main cropping system in the south of China [1]. An increase in chemical fertilizer for sustained high yield has led to the loss of soil organic carbon and biodiversity, thus risking stable crop yields and reducing soil microbial diversity [2-5]. With renewed interest in

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maintaining soil health and clean production on farmland, crop rotation may be an effective solution to establish a diversified planting system that can stabilize crop yields and increase soil carbon pools and biodiversity [6].

Crop rotation, as a nature-based solution, has been proven to i) increase farmland biodiversity, thus decreasing pest and disease incidence, and maintaining crop yield stability [7]; ii) reduce soil erosion because of plant cover [8], thus protecting soil nutrients in the cultivated layer of farmland; iii) maintain soil temperature and reduce water dissipation, thus improving soil hydrothermal environment [9]; and iv) affect the decomposition and transformation of soil organic carbon through the input of root residues and release of root exudates, thus enhancing soil organic carbon pools and optimizing soil microbial community structure and diversity [10,11].

Crop rotation can enhance crop yields and soil carbon sequestration, but their contribution differs depending on the combination of crops [12]. Milk vetch, a leguminous crop, can improve rice productivity, with reduced N fertilizer input and total global warming potential (GWP) per yield [13,14]. Rape, a brassica crop, may increase soil gross N immobilization rates and decrease soil gross N mineralization rates in a double rice cropping system [15]. In the upland soil, the wheat–soybean rotation can result in the greatest richness and biodiversity of the total microbial community [16]. Moreover, various crop rotations have been selected for different microbial communities, which have generated microbial products that significantly influenced soil C and N retention [17,18]. Relatively small increases in crop diversity could have large impacts on microbial community size and function, with cover crops appearing to facilitate the largest increases [19]. However, it is still not clear what winter crop rotation is suitable for this task in the double rice field and how to intensify rotation with various winter crops.

Thus, a better understanding is needed for what winter crop rotation in double rice fields, especially rotation with various winter crops, may contribute to crop yield, and how they can provide positive feedback in soil carbon and microbes. In this study, we characterized the crop yield, soil carbon, nitrogen, and soil microorganisms under various winter crop treatments in double rice fields. The main aim of this study was to investigate the effects of diverse crop rotation practices on crop yield, soil C, N, and microbes. It was hypothesized that winter crop rotation intensification in double rice fields can increase crop yield, be effective in soil carbon sequestration and nitrogen content, and improve soil microbial structure and diversity.

2. Materials and methods

2.1. Experimental site

The field experiment was conducted from 2012 to 2019 at the Agricultural Science Institute of Wannian County (28°42′N, 117°04′E), Jiangxi, China. The region belongs to the subtropical monsoon climate, with abundant sunlight, heat, and precipitation resources and significant seasonal changes in temperature. The mean monthly temperature and precipitation during the experiment are shown in Fig. 1. The experimental soil is a typical double rice paddy field in the middle and lower reaches of the Yangtze River. Before the study, the soil was hydromorphic paddy soil with 6.08 pH, 41.81 g kg^{-1} of soil organic matter, 1.97 g kg^{-1} of total nitrogen,
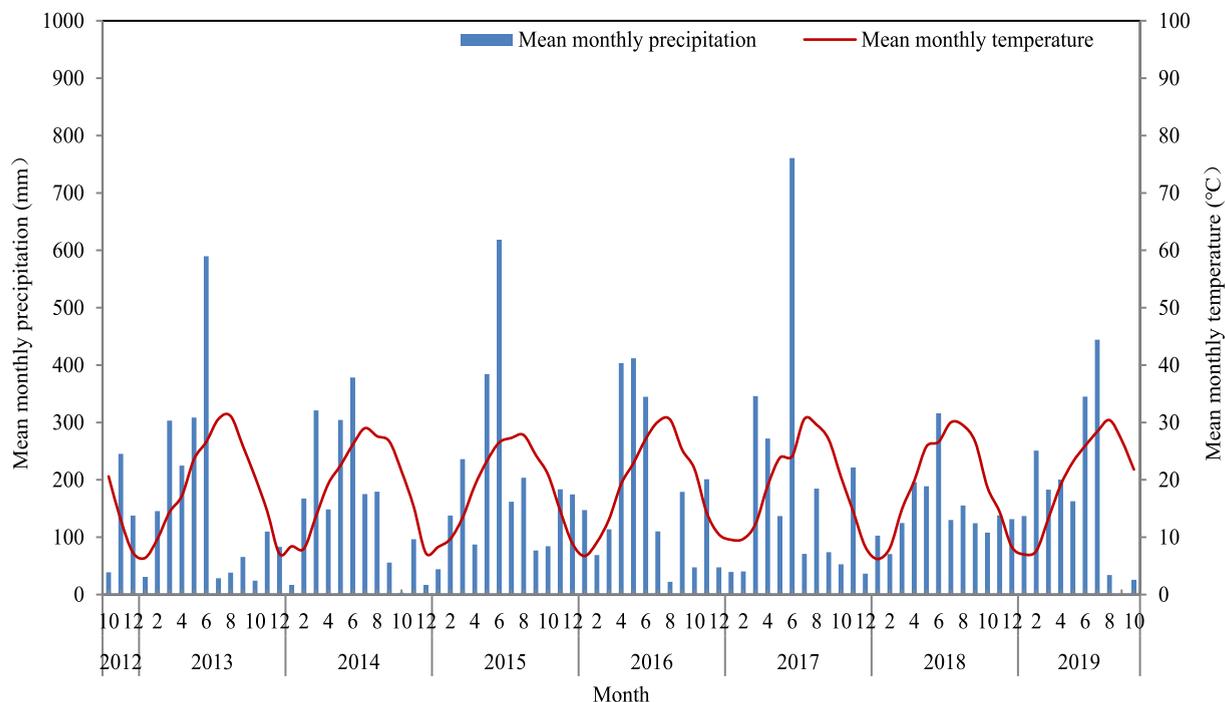

Fig. 1. Mean monthly temperature and precipitation during the experiment.

Table 1
Experimental design.

Treatments	2012.10–2013.10	2013.10–2014.10	2014.10–2015.10	2015.10–2016.10	2016.10–2017.10	2017.10–2018.10	2018.10–2019.10
T0 (winter fallow)	fallow - double rice	fallow - double rice	fallow - double rice	fallow - double rice	fallow - double rice	fallow - double rice	fallow - double rice
T1 (winter milk vetch)	milk vetch- double rice	milk vetch - double rice	milk vetch - double rice	milk vetch - double rice	milk vetch - double rice	milk vetch - double rice	milk vetch - double rice
T2 (winter rape)	rape - double rice	rape - double rice	rape - double rice	rape - double rice	rape - double rice	rape - double rice	rape - double rice
T3 (winter garlic)	garlic - double rice	garlic - double rice	garlic - double rice	garlic - double rice	garlic - double rice	garlic - double rice	garlic - double rice
T4 (winter crop rotation)	potato - double rice	milk vetch- double rice	rape - double rice	potato - double rice	milk vetch- double rice	rape - double rice	potato - double rice

16.38 mg kg^{-1} of available phosphorus, and 130 mg kg^{-1} of available potassium.

2.2. Experimental design

The long-term field experiment consisted of five cropping systems based on double rice. T0: winter fallow, a traditional cropping system, was used as a control; T1: winter milk vetch, a green manure cropping system; T2: winter rape, an oil-bearing cropping system; T3: winter garlic, a vegetable cropping system; T4: winter potato-milk vetch-rape, a cover crop rotation intensification system. The whole experimental design consisted of five treatments (T0, T1, T2, T3, and T4) with a randomized block design and three replications in each treatment. There were 15 experimental plots in total, each with an area of 66 m^2 (11 m × 6 m). The experimental design of the annual planting pattern is detailed in Table 1. Taking winter crop rotation treatment (T4) as a reference, the parameters of the third (2015) and sixth (2018) years were mainly used for analysis in this study.

The cultivar of milk vetch was Yujiang Daye, which was sown (45 kg·hm^{-2} seeds) in October and harvested as green manure in March. The cultivar of rape was Rongyou 05 in 2012–2014, Ganyouza 8 in 2015, and Mianfengyou 18 in 2016–2018, which was sown (23 kg·hm^{-2} seeds) in October and harvested in May. The cultivar of garlic was Jinxiang Dasuan, which was sown (2500 kg·hm^{-2} seeds) in November and harvested in March. The cultivar of potato was Kexin 18 in 2012 and 2015, and Dongnong 303 in 2018, which was sown (2000 kg·hm^{-2} seeds) in November and harvested in April. The cultivar of early rice was Tianyou 463 in 2013, Zhuliangyou 09 in 2014, 2016–2019, and Xinrong 08 in 2015, which was transplanted in May and harvested in July. The cultivar of early rice was Tianyou Huazhan in 2013–2019, which was transplanted in July and harvested in October. Fertilizer was applied according to local conventional fertilization, and other field management measures were the same as general field cultivation.

2.3. Sampling and analysis of crop

A sampling of rice crop yield was carried out each year (2013–2019) and sampling of rice crop yield components and dry matter weight was carried out in 2015 and 2018. The panicle length, seed setting rate, effective panicle number, thousand-grain weight, and yield of rice were measured at the harvest stage. The yield of rice was air-dried to constant weight. The shoot biomass of rice was also measured at the tillering stage, booting stage, heading stage, and harvest stage, dried at 105 °C for 30 min and then dried at 80 °C to constant weight.

2.4. Soil carbon and nitrogen

A sampling of soil was carried out at the harvest stage of rice in 2015 and 2018. Five representative soil samples were taken with the help of an auger from each replicate of the individual treatments to a depth of 15 cm and then combined to form a composite sample. All samples were sieved through a 0.25 mm sieve after air-drying. The air-drying soil samples were used to analyze soil organic carbon (SOC) and total nitrogen (TN) concentrations. SOC concentrations were analyzed by combustion analysis (TOC-L SSM-5000A, SHIMADZU). TN concentration was analyzed by Kjeldahl determination (Kjeltec™ 2300, FOSS).

2.5. Soil microorganisms

Sampling for soil microbes was collected from each plot in April 2019 before the winter crop harvest. Fifteen soil samples were taken with the help of an auger from each plot to a depth of 15 cm. All samples were individually removed from roots and stones, and then a portion of each fresh mixed sample was bagged with a sterile spoon, placed in ice buckets to bring them back to the laboratory, and stored at −80 °C immediately for measuring microorganisms.

Total genomic DNA in the soil samples was extracted using the CTAB method. DNA concentration and purity were detected in 1% agarose gels, and DNA was diluted to 1 ng μL^{-1} using sterile water. Bacterial 16S rRNA gene fragments from the V4 region were amplified by a PCR method using the universal primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) [20]. All PCRs were conducted with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). The same quantity of 1 × loading buffer (containing SYB green) was mixed with the PCR products according to their concentrations, and the samples were subjected to electrophoresis on 2% agarose gels for detection. Samples with a bright main band around 300 bp for bacteria, were selected for further examination. The target strips were purified using a Gel Extraction Kit (Qiagen). Sequencing libraries were built using a TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA). The qualified libraries were assessed using a Qubit@ 2.0 Fluorometer (Thermo Scientific) as well as PCR quantitative detection and were sequenced using a HiSeq 2500 PE250 platform by the Novogene Company (Beijing, China). The sequencing data has been uploaded to the National Center for Biotechnology Information (NCBI) database, and the BioProject accession number is PRJNA911586.

2.6. Data statistics

Statistical analysis of all experimental data was conducted using SPSS 17.0 and Microsoft EXCEL 2010. Crop yield, soil carbon, nitrogen, and microbe data were averaged for each treatment and were evaluated with one-way ANOVA. The mean values of all data in different treatments were compared using Duncan's multiple comparisons for each sampling point. The significant difference was assessed at $P < 0.05$. Species abundance information was normalized using a standard of sequence number corresponding to the sample with the least sequences. Subsequent analysis of alpha diversity and beta diversity were all performed based on this output

normalized data. Alpha diversity is applied in analyzing the complexity of species diversity for a sample through 2 indices, including Shannon and Simpson's. All these indices in our samples were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3). Cluster analysis was preceded by principal component analysis (PCA), which was applied to reduce the dimension of the original variables using the FactoMineR package and ggplot2 package in R software (Version 2.15.3). The unweighted pair-group method with arithmetic means (UPGMA) clustering was performed as a type of hierarchical clustering method to interpret the distance matrix using average linkage and was conducted by QIIME software (Version 1.7.0).

3. Results

3.1. Crop yield

Rice yields under the winter garlic and crop rotation treatments were consistently at high levels, while rice yields under the winter fallow and rape treatments were relatively low (Fig. 2a and b). The results of the 7-year average rice yield showed that early rice yield was 4.6% and 3.5% higher in winter garlic and crop rotation treatments than in winter fallow, respectively; and late rice yield was 2.5%, 2.3%, 4.5%, and 3.7% higher in winter milk vetch, rape, garlic, and crop rotation treatments than in winter fallow, respectively (Fig. 2c). Winter cover crops can significantly increase the rice yield, among which the most significant effects of winter garlic and crop rotation treatments on rice yield were observed.
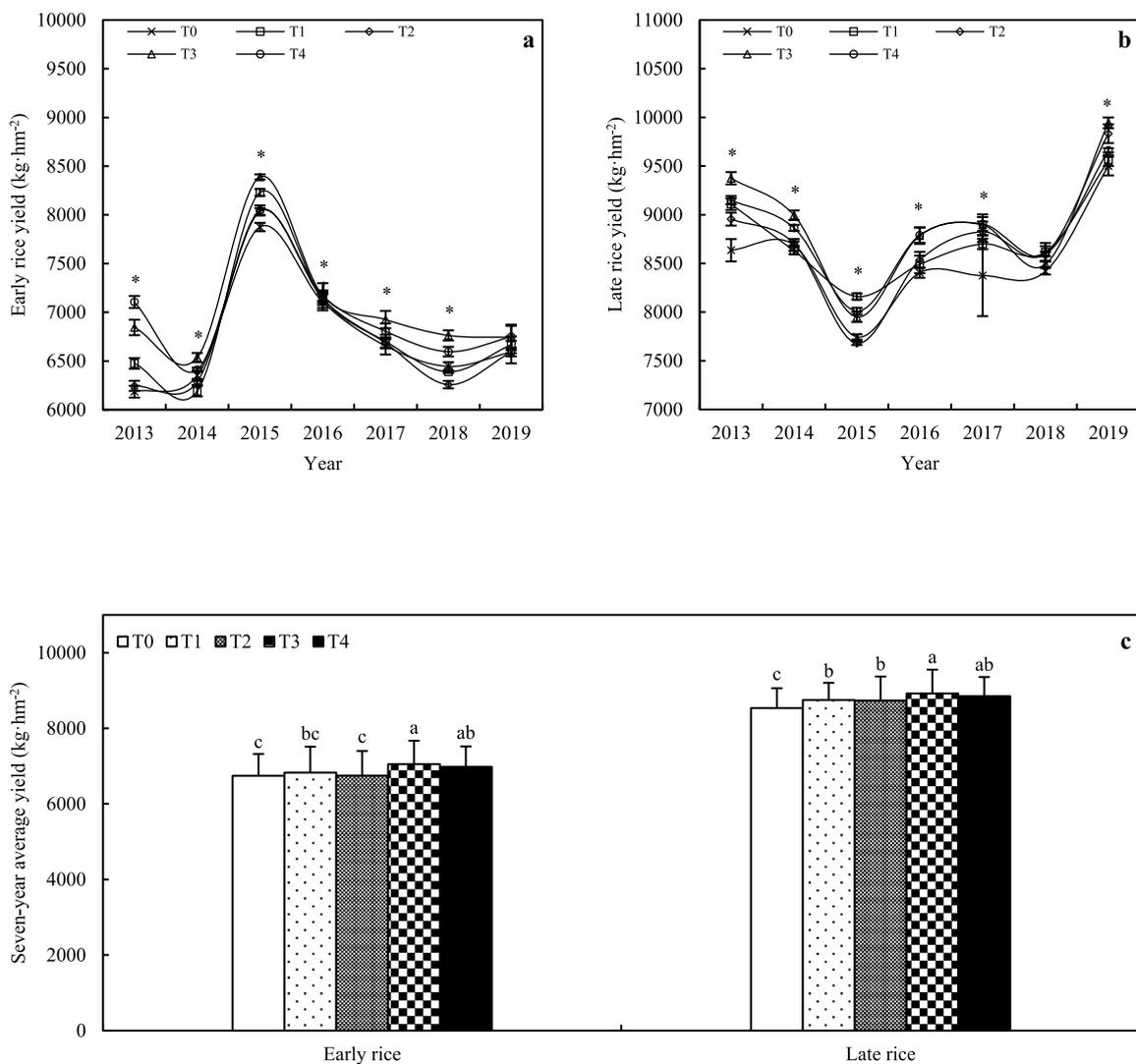

Fig. 2. Effects of winter cover crops on rice crop yield. *Note:* Fig. 2a and b represent the rice yield in each year under different cover crops (Means of three replicates and standard deviations). Fig. 2c represents the seven-year average rice yield under different cover crops (Means of seven years and standard deviations). The asterisks and letters above means indicate significant differences at $P < 0.05$.

3.2. Crop yield components

The effects of different winter crops on panicle length, seed setting rate, and thousand-grain weight of double rice were not significant, but the effects on effective panicle number were significant (Table 2). In 2015, the effective panicle number of early rice in winter milk vetch, garlic, and crop rotation significantly increased by 4.6%, 6.7%, and 4.0%, respectively, compared with winter fallow, and the effective panicle number of late rice in winter milk vetch, garlic, and crop rotation significantly increased by 3.1%, 3.1%, and 2.8% compared with winter fallow. In 2018, the effective panicle number of early rice in winter milk vetch and crop rotation also significantly increased by 26.0% and 32.0%, respectively, compared with winter fallow. It can be observed that winter crops increase rice yield mainly by enhancing the effective panicle number.

3.3. Crop dry matter weight

The effect of different winter crops on the dry matter weight of early and late rice varied (Table 3). In 2015, winter crops mainly affected the dry matter weight of early rice, whereas winter garlic significantly improved the dry matter weight of early rice by 24.6%, 17.0%, and 12.2% at the booting, heading and harvest stages, respectively, compared with winter fallow, while it had some effect on the dry matter weight of late rice at the tillering stage. In 2018, the effect of winter crops on the dry matter weight of early and late rice was weak, whereas winter garlic and crop rotation only significantly improved the dry matter weight of early rice by 16.5% and 20.0%, respectively, at the heading stage compared with winter fallow. Therefore, the effect of winter crops on the dry matter weight of rice was found to be limited.

3.4. Soil carbon and nitrogen

Winter crops can significantly affect soil carbon and nitrogen (Table 4). In 2015, winter crops significantly increased the total soil organic carbon content by 7.3%–11.6% and winter garlic increased the total soil nitrogen content by 20.5% in the late rice season compared with winter fallow. In 2018, winter crops significantly increased the total soil organic carbon content by 6.1%–7.2% and winter milk vetch, garlic and winter crop rotation significantly increased the total soil nitrogen content by 12.8%, 7.3%, and 10.4%, respectively, in the early rice season compared with winter fallow. At the same time, winter garlic and winter crop rotation also significantly increased the total soil organic carbon content by 19.3% and 20.8%, respectively, in the late rice season compared with winter fallow. This indicates that winter crops promote the accumulation of soil organic carbon, which is especially stable and obvious with winter crop rotation.

3.5. Soil microbial community abundance

Among the studied phylum classifications, Proteobacteria accounted for 34.83%–50.97%, Acidobacteria for 10.26%–15.50%, Bacteroidetes for 9.03%–12.93%, Nitrospirae for 4.73%–12.45%, and Gemmatimonadetes for 4.33%–7.40% (Fig. 3a). The various winter crops significantly increased the abundance of Proteobacteria and Bacteroidetes, and significantly decreased the abundance of Acidobacteria and Nitrospirae compared to the winter fallow. Winter crops significantly increased the abundance of bacterial phyla in the order of T2 > T4 > T3 > T1 > T0, by 3.39%, 2.87%, 2.64%, and 1.75%, respectively, compared to the winter fallow.

Table 2
Effects of winter cover crops on rice crop yield components.

Year	Rice season	Treatment	Panicle Length (cm)	Seed setting rate (%)	Effective panicle number (10^4 hm^{-2})	Thousand grain weight (g)
2015	Early rice	T0	20.54 ± 0.24a	86.23 ± 0.65b	298.45 ± 0.82c	25.82 ± 0.06a
		T1	21.41 ± 0.38a	85.77 ± 0.41b	312.23 ± 1.86b	26.12 ± 0.33a
		T2	21.21 ± 0.28a	88.48 ± 1.31 ab	300.91 ± 2.02c	26.14 ± 0.38a
		T3	21.39 ± 0.32a	89.10 ± 0.05a	318.58 ± 2.14a	26.00 ± 0.21a
		T4	20.70 ± 0.47a	86.78 ± 1.13 ab	310.48 ± 1.21b	26.10 ± 0.23a
	Late rice	T0	20.25 ± 0.19a	83.03 ± 0.40a	304.18 ± 3.24b	25.20 ± 0.35a
		T1	21.15 ± 0.29a	85.71 ± 0.90a	313.74 ± 1.25a	26.06 ± 0.39a
		T2	20.77 ± 0.44a	83.13 ± 0.63a	309.34 ± 0.24 ab	25.91 ± 0.12a
		T3	20.70 ± 0.36a	84.86 ± 1.21a	313.65 ± 2.34a	25.01 ± 0.47a
		T4	20.50 ± 0.08a	84.52 ± 0.60a	312.82 ± 2.09a	25.65 ± 0.03a
2018	Early rice	T0	17.43 ± 0.72a	81.86 ± 1.19a	296.67 ± 0.67c	23.61 ± 0.25a
		T1	16.54 ± 1.33a	82.33 ± 0.65a	373.80 ± 1.15b	23.65 ± 0.35a
		T2	18.06 ± 0.23a	81.94 ± 1.91a	332.27 ± 0.88bc	23.45 ± 0.61a
		T3	17.66 ± 0.45a	84.39 ± 2.03a	320.40 ± 1.15bc	24.37 ± 0.45a
		T4	17.88 ± 0.28a	84.99 ± 1.84a	391.60 ± 0.58a	24.44 ± 0.12a
	Late rice	T0	22.79 ± 0.44a	89.68 ± 0.82a	315.00 ± 12.60a	23.9838 ± 0.44a
		T1	23.58 ± 0.34a	89.93 ± 2.43a	346.50 ± 44.10a	24.0645 ± 0.30a
		T2	22.75 ± 0.02a	90.29 ± 0.42a	327.60 ± 6.30a	24.1157 ± 0.26a
		T3	23.34 ± 0.54a	90.80 ± 1.91a	327.6 ± 35.08a	24.1306 ± 0.56a
		T4	23.16 ± 0.47a	90.58 ± 0.64a	384.30 ± 12.60a	24.2719 ± 0.09a

Note: Means of three replicates and standard deviations. Letters indicate significant differences between the crops at *P* < 0.05.

Table 3
Effects of winter cover crops on rice crop dry matter weight.

Year	Rice season	Treatment	Tillering stage (10^3 kg hm^{-2})	Booting stage (10^3 kg hm^{-2})	Heading stage (10^3 kg hm^{-2})	Harvest stage (10^3 kg hm^{-2})
2015	Early rice	T0	1.3 ± 0.10a	3.05 ± 0.16b	5.30 ± 0.07c	11.72 ± 0.38b
		T1	1.65 ± 0.07a	3.58 ± 0.18ab	5.90 ± 0.13ab	12.43 ± 0.24ab
		T2	1.62 ± 0.14a	3.29 ± 0.10ab	5.78 ± 0.06b	12.98 ± 0.23ab
		T3	1.76 ± 0.28a	3.80 ± 0.19a	6.20 ± 0.15a	13.15 ± 0.40a
		T4	1.56 ± 0.08a	3.37 ± 0.21ab	5.71 ± 0.06b	11.96 ± 0.57ab
	Late rice	T0	3.33 ± 0.05b	6.02 ± 0.68a	8.73 ± 0.12a	13.91 ± 0.03a
		T1	3.52 ± 0.20 ab	5.87 ± 0.67a	9.08 ± 0.31a	15.66 ± 0.78a
		T2	3.47 ± 0.02 ab	6.82 ± 0.10a	8.94 ± 0.19a	14.33 ± 0.39a
		T3	3.86 ± 0.19a	6.97 ± 0.26a	9.07 ± 0.22a	15.24 ± 0.92a
		T4	3.40 ± 0.03b	6.70 ± 0.06a	8.98 ± 0.08a	14.73 ± 0.62a
2018	Early rice	T0	1.26 ± 0.12a	1.69 ± 0.14a	4.49 ± 0.23b	7.35 ± 0.32a
		T1	1.33 ± 0.16a	1.82 ± 0.24a	5.01 ± 0.20ab	8.25 ± 0.81a
		T2	1.37 ± 0.11a	1.82 ± 0.12a	4.52 ± 0.29b	7.57 ± 0.52a
		T3	1.40 ± 0.09a	2.11 ± 0.09a	5.23 ± 0.08a	8.25 ± 0.14a
		T4	1.59 ± 0.10a	2.08 ± 0.16a	5.39 ± 0.16a	8.73 ± 0.21a
	Late rice	T0	4.20 ± 0.18a	6.51 ± 0.35a	9.18 ± 0.31a	12.19 ± 0.22a
		T1	4.35 ± 0.21a	6.69 ± 0.36a	9.39 ± 0.21a	12.48 ± 0.57a
		T2	4.31 ± 0.58a	6.78 ± 0.34a	9.40 ± 0.22a	12.50 ± 0.16a
		T3	4.55 ± 0.29a	7.19 ± 0.63a	9.42 ± 0.28a	12.45 ± 0.34a
		T4	4.54 ± 0.37a	6.89 ± 0.49a	9.58 ± 0.43a	13.20 ± 0.84a

Note: Means of three replicates and standard deviations. Letters indicate significant differences between the crops at $P < 0.05$.

Table 4
Effects of winter cover crops on soil carbon and nitrogen.

Rice season	Treatment	2015		2018	
		SOC/(g·kg^{-1})	TN (g·kg^{-1})	SOC/(g·kg^{-1})	TN (g·kg^{-1})
Early rice	T0	20.85 ± 0.07a	1.20 ± 0.07a	20.91 ± 0.13b	1.64 ± 0.01c
	T1	21.00 ± 0.88a	1.23 ± 0.04a	22.23 ± 0.25a	1.85 ± 0.05a
	T2	21.07 ± 0.73a	1.22 ± 0.06a	22.19 ± 0.27a	1.71 ± 0.01bc
	T3	21.64 ± 1.30a	1.24 ± 0.11a	22.22 ± 0.21a	1.76 ± 0.04ab
	T4	21.99 ± 1.43a	1.23 ± 0.00a	22.41 ± 0.12a	1.81 ± 0.01ab
Late rice	T0	20.45 ± 0.22b	1.32 ± 0.06b	18.14 ± 1.43b	1.40 ± 0.07a
	T1	22.82 ± 1.13a	1.34 ± 0.03b	20.08 ± 0.88ab	1.49 ± 0.05a
	T2	22.11 ± 1.06a	1.44 ± 0.12b	20.35 ± 0.23ab	1.44 ± 0.07a
	T3	22.35 ± 0.71a	1.59 ± 0.06a	21.65 ± 0.22a	1.45 ± 0.05a
	T4	21.94 ± 0.57a	1.39 ± 0.03b	21.91 ± 0.43a	1.50 ± 0.07a

Note: Means of three replicates and standard deviations. Letters indicate significant differences between the crops at $P < 0.05$.

The highest relative abundance of unidentified_Rokubacteria, Geobacter, and unidentified_Deltaproteobacteria was found in the winter fallow treatment; Methylotenera, Pseudomonas, and Mycobacterium in the winter garlic treatment; and Cellvibrio and Bryobacter in the winter crop rotation treatment (Fig. 3b). Observed at the genus level, species abundance varied greatly between winter crops and winter fallow treatments, with winter garlic and crop rotation being the most pronounced.

3.6. Soil microbial community composition

The results of PCA analysis based on OTU levels showed that the soil microbial community composition differed significantly between the treatments (Fig. 4a). The soil microbial community composition under winter crops showed a large divergence in PC1 and PC2 compared to the winter fallow, which demonstrated the strong influence of winter crops on the soil microbial community. Large differences were also shown between winter milk vetch and garlic, while the differences between winter rape and crop rotation were smaller.

Cluster analysis at the phylum level revealed that the different treatments eventually clustered into two categories, one for the winter fallow treatment and one for the winter crop treatment, where winter milk vetch and crop rotation clustered into one category and winter rape and garlic clustered into another category among the winter crop treatments (Fig. 4b). It further showed that there was a great difference between the soil microbial community composition of winter fallow and crops.

3.7. Soil microbial community alpha diversity

The soil microbial community alpha diversity analysis between different treatments revealed that the Shannon diversity index of winter crops ranged from 9.75 to 9.91, while that of winter fallow was 9.38, and that of winter crops was in all cases extremely
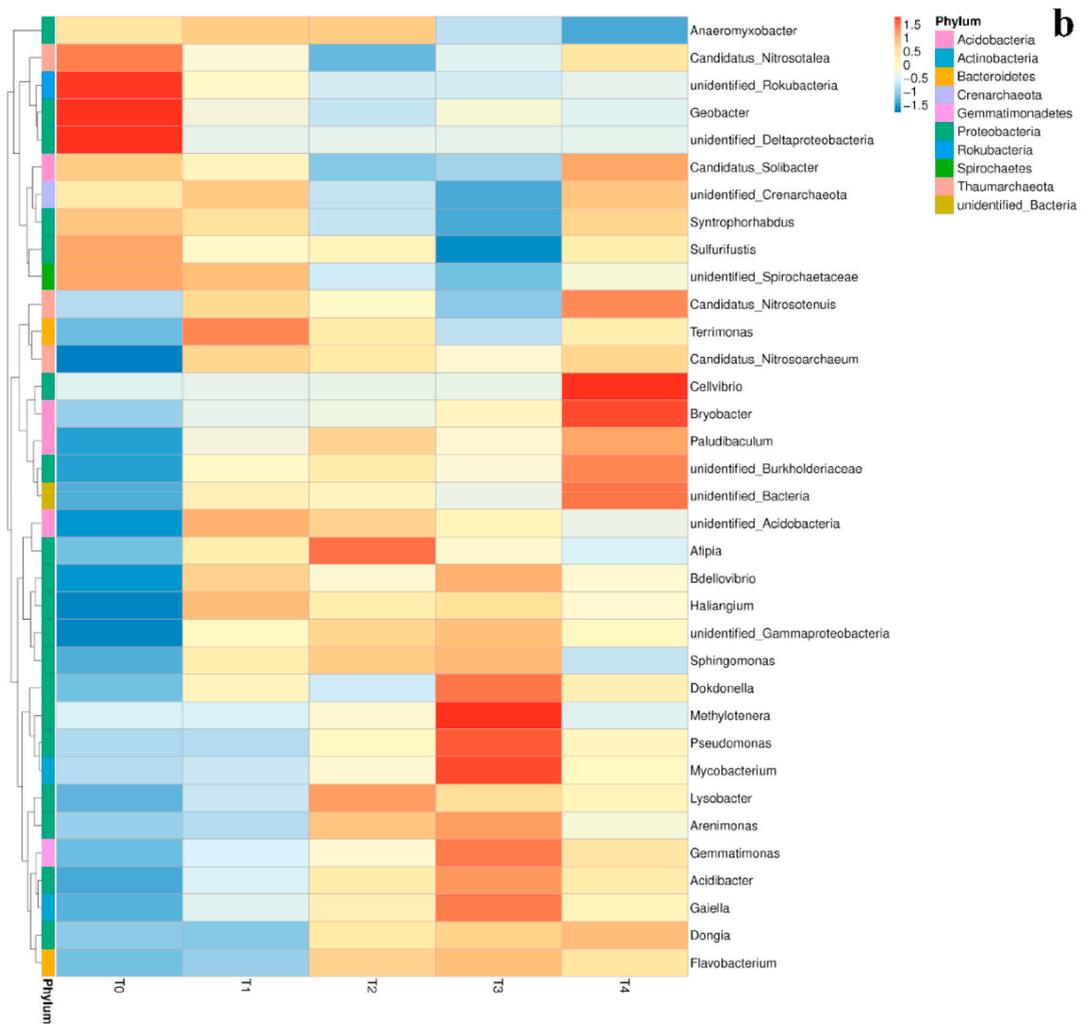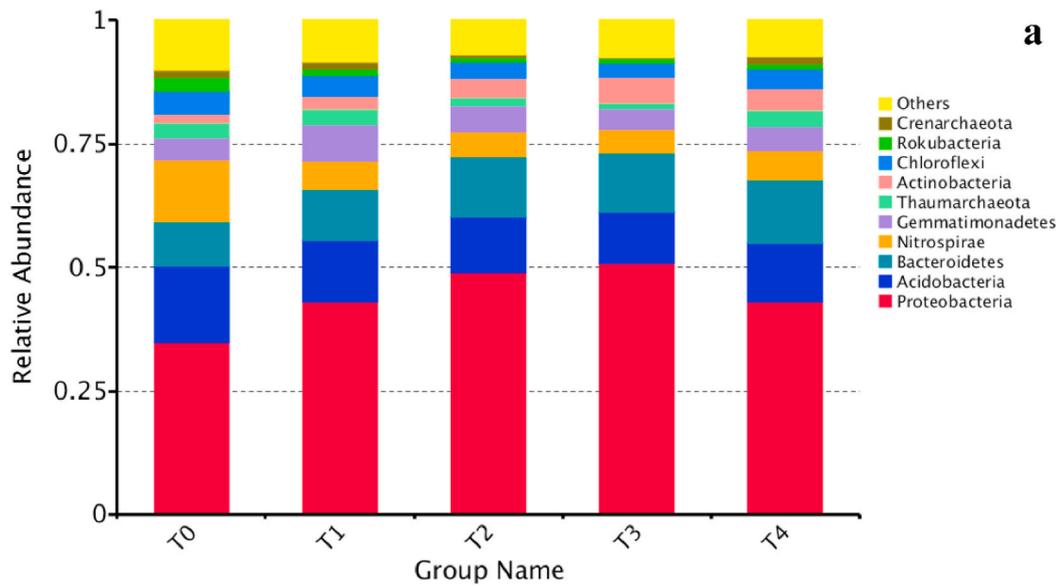
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Fig. 3. Effects of winter cover crops on the relative abundance of soil bacteria at the phylum and genus level. *Note:* Fig. 3a shows the histogram of the relative abundance of soil bacteria at the phylum level. The horizontal coordinate indicates the treatments of different winter cover crops; the vertical coordinate indicates the relative abundance of soil bacteria. Proteobacteria, Acidobacteria, Bacteroidetes, Nitrospirae, Gemmatimonadetes, Thaumarchaeota, Actinobacteria, Chloroflexi, Rokubacteria, and Crenarchaeota were the top 10 dominant bacteria at the phylum level. Others were the sum of the relative abundances of all other phyla. Fig. 3b shows the clustering map of the relative abundance of soil bacteria at the genus level. The top 35 dominant genera were selected based on bacteria annotation and abundance information of all samples at the genus level. Vertical is the sample information, horizontal is the bacteria annotation information, and the clustering tree on the left side of Fig. 3b is the bacteria clustering tree.

significantly higher than that of winter fallow (Fig. 5a). Furthermore, the Simpson's diversity index of winter crops ranged from 0.997 to 0.998, while that of winter fallow was 0.996, and that of winter crops was in all cases also extremely significantly higher than that of winter fallow (Fig. 5b). The results showed that winter crops significantly increased soil microbial community diversity in a double rice field.

4. Discussion

Compared to winter fallow, winter milk vetch, rape, garlic, and crop rotation practice increased the late rice yield by 2.5%, 2.3%, 4.5% and 3.7%, respectively, and winter garlic and crop rotation also increased the early rice yield by 4.6% and 3.5%, respectively (Fig. 2). This illustrated that winter crop rotation can improve late rice yield, and winter garlic and crop rotation also have advantages in enhancing early crop yield. The increase in rice yield was directly attributed to the fact that the winter crop promoted rice tillering, thus increasing the effective panicle number of rice (Table 2). Furthermore, the indirect reason for the increase in crop yield may be that winter crops increase farmland biodiversity, soil carbon, and nitrogen content [16,21], thus improving soil microbial structure and increasing soil microbial diversity [19,22,23].

Legume winter crop (milk vetch) can apply more nitrogen by N-fixing for the subsequent crop [24]; non-legume winter crops (rape, garlic, potato) are most useful for suppressing weeds [25], controlling the loss of soil nutrients [26] and inputting crop residue [27] that can add soil organic carbon content; legume and non-legume cover crop rotation over many years further increases the biodiversity of farmland, and the input of crop residue will be more diversified, which can regulate the decomposition of high and low-quality residues [28] and provide a more balanced soil carbon and nitrogen supply [29], thus being more conducive to the regulation of soil C/N ratio [27]. This is why, compared to winter fallow, winter crop rotation intensification practice increased the soil organic carbon and total nitrogen content by 21% and 7%, respectively (Table 4).

Winter crops, compared to winter fallow, increased the abundance of bacteria (Fig. 3), changed soil microbial community composition (Fig. 4), and increased soil microbial community diversity (Fig. 5). Generally, soil microbial abundance and diversity were closely associated with soil environment changes: pH, temperature, moisture, cation exchange capacity, C/N ratio, crop residues, and soil organic carbon [30]. In this study, the rotation of various winter crops input diversified crop residues and released more kinds of root exudates, thus changing soil microbial structure and diversity [31]. Soil microbial diversity was found to be positively correlated with crop yields [32], potentially leading to more-efficient processing of soil nutrients and thus more nutrient uptake and subsequently higher yields. Therefore, our results show that winter cover crop rotation, as a biodiversity solution in double rice fields, can increase soil carbon sequestration, regulate soil C/N ratio, and improve soil microbial structure and diversity, which can keep soil healthy and sustainable, thus promoting rice tillering and enhancing crop yield stability.

Finally, we have only set up one winter crop rotation system in this study, which is still not enough to fully prove its advantages. In the future, it is necessary to design more diversified combinations of winter crop rotations, which will provide a better understanding of how to rotate with diversified cover crops and provide greater insight into their effectiveness.

5. Conclusions

  i) Winter crops can increase double rice yield, which is associated with the promotion of rice tillering.
  ii) Winter crops with the input of diversified residues, compared to winter fallow, contribute to soil carbon sequestration, improve soil microbial structure, and increase soil microbial diversity.
  iii) Winter crop rotation was not only 3.5% (early rice) and 3.7% (late rice) higher in terms of crop yield than winter fallow, but the soil organic carbon content also increased by 21%.

In conclusion, winter crop practices, especially winter crop rotation intensification, can largely keep soil healthy and sustainable for double rice fields through its positive feedback in terms of crop yield, soil carbon and microorganism.

Author contribution statement

QUAN ZHOU: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Peng Zhang: Performed the experiments; Analyzed and interpreted the data.

Zhiqiang Wang; Lixian Wang: Performed the experiments.

Shubin Wang; Guoqin Huang: Conceived and designed the experiments.

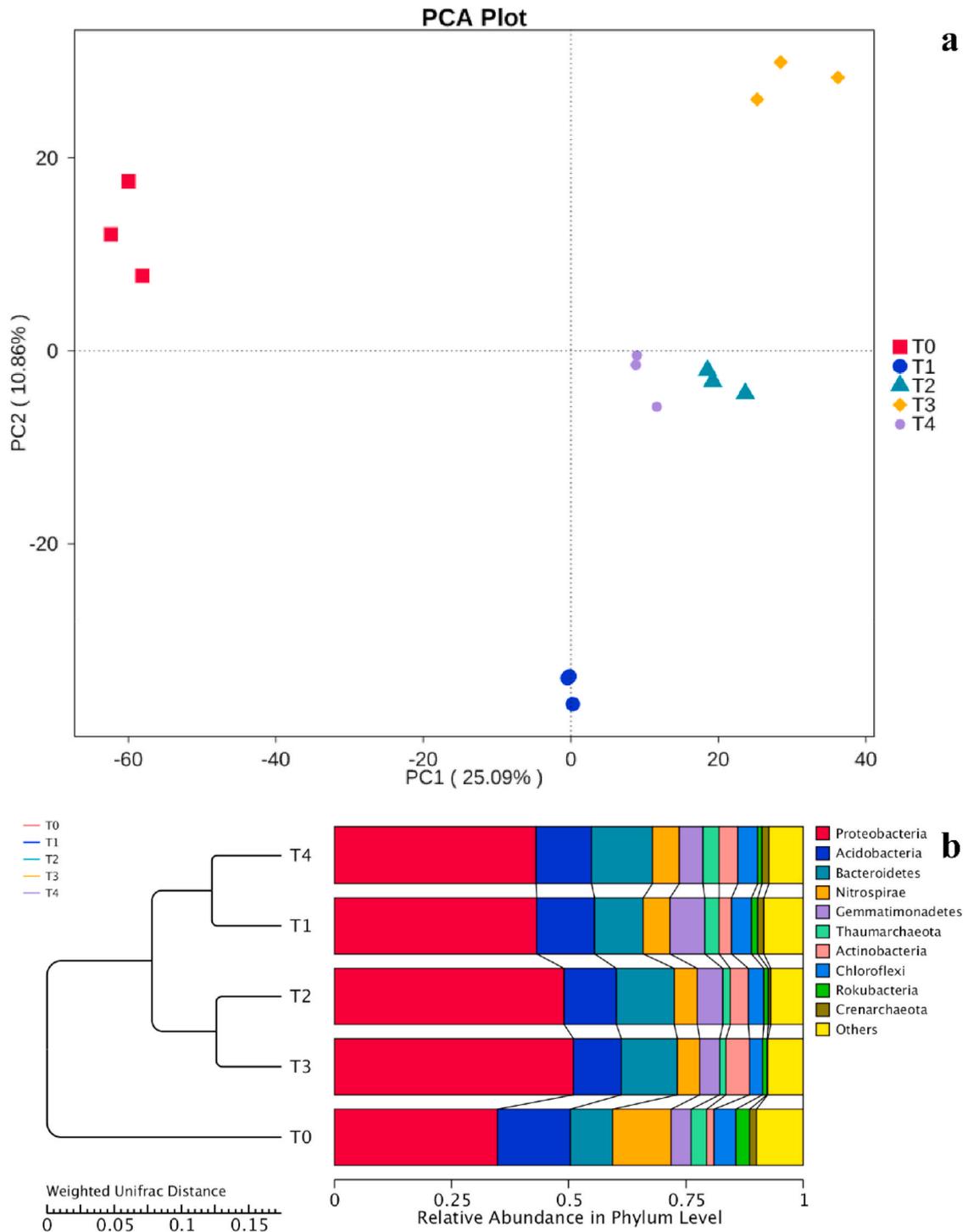Wenting Yang; Binjuan Yang: Contributed reagents, materials, analysis tools or data.

Fig. 4. PCA and cluster analysis for soil microbial community composition under different winter cover crops. *Note:* Fig. 4a shows PCA scores for soil microbial community composition. PCA was performed on soil microbial community composition based on OTU levels. Percentage values in brackets indicate the variation explained by the principal components. Fig. 4b shows cluster analysis for soil microbial community composition. The clustering tree on the left side of Fig. 4b is based on the weighted unifrac distance UPGMA (Unweighted Pair-group Method with Arithmetic Mean). The right side of Fig. 4b shows the distribution of the relative abundance of soil bacteria at the phylum level for each treatment.

(caption on next page)

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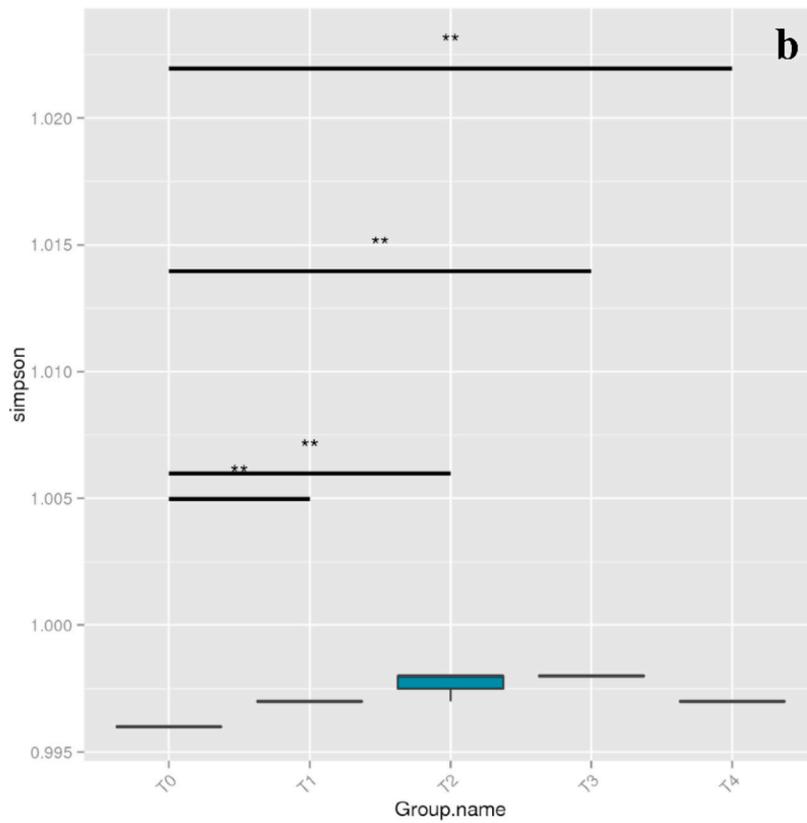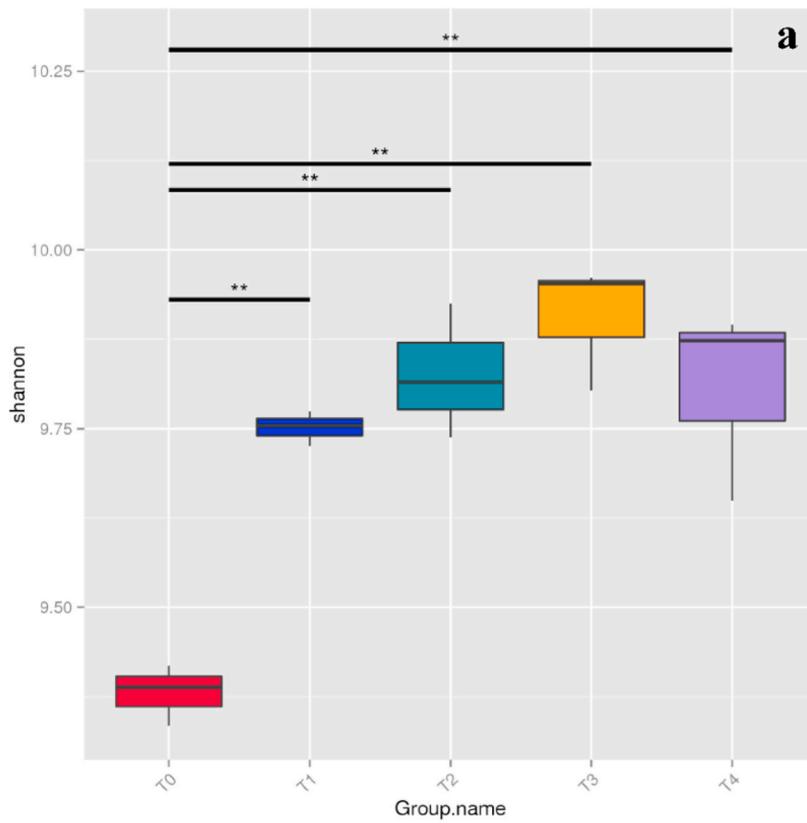
Fig. 5. Effects of winter cover crops on soil microbial community diversity. *Note:* Fig. 5a represents the Shannon diversity index under different treatments. Fig. 5b represents the Simpson diversity index under different treatments. Means of three replicates and standard deviations. The double asterisks above means indicate extremely significant differences at $P < 0.01$.

Data availability statement

The authors do not have permission to share data.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

T0	Winter fallow
T1	Winter milk vetch
T2	Winter rape
T3	Winter garlic
T4	Winter rotation intensification with potato, milk vetch and rape
C	Carbon
SOC	Soil organic carbon
N	Nitrogen
TN	Total nitrogen
GWP	Global warming potential
PCA	Principal component analysis

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