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Effect of the Different Fertilization Treatments Application on Paddy Soil Enzyme Activities and Bacterial Community Composition

Qingqing Xiao [1,*], Boping He [1] and Su Wang [2,*]

[1] School of Biology, Food and Environment, Hefei University, Hefei 230601, China
[2] College of Art, Anhui University, Hefei 230601, China
* Correspondence: xiaoqq@hfuu.edu.cn (Q.X.); wangsu@ahu.edu.cn (S.W.)

Abstract: The application amount of fertilizer is believed to dramatically influence soil bacteria in paddy fields, and soil bacteria critically affect soil enzyme activities and the growth of rice in paddy fields. Thus, providing a suitable amount of fertilization to ensure rice yields is an important issue in field management. In this study, four treatments were carried out in a paddy field, including control (fertilization methods commonly used by farmers in the past), IT + RF 10, IT + RF 20, and IT + RF 30 (integrated technology and 10%, 20%, and 30% reduction in nitrogen fertilizer, respectively). Soil samples were collected in two periods, the rice booting stage and the harvest period. High-throughput sequencing was used for analysis of soil bacterial diversity and community composition across different fertilizer treatments, and clarified the effects of different fertilizer treatments on soil enzyme activities and bacterial community composition. This study showed that a reduction in chemical fertilizer application has a certain impact on the soil pH, total nitrogen (TN), total phosphorus (TP), soil organic matter (SOM) value, and bacterial community of the rice planting system, and that the IT + RF 10 treatment was the best way to reduce fertilizer application, which can reduce nutrient loss in the paddy soil. The application of organic fertilizer partially replaces chemical fertilizer, which not only effectively stimulates soil enzyme activity, but also enriches bacterial groups that may participate in complex organic matter decomposition and soil nutrient mobilization.

Keywords: soil enzyme activities; soil bacterial diversity; 16S rRNA sequencing; bacterial community structure; fertilizer reduction

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1. Introduction

Rice is widely grown worldwide and is a staple food for more than half of the world's population. High yield of rice requires rational fertilization strategies and abundant soil fertility. However, farmers continue to increase the use of chemical fertilizers in order to increase rice yields. Excessive use of fertilizers will have a great impact on soil microorganisms and reduce the utilization rate of nutrients in fertilizers, which is not conducive to the sustainable development of agriculture [1,2]. Soil microbes are abundant in agricultural soil environments and play an important role in agricultural ecosystems, which are easily affected by environmental changes [3]. One typical method of changing the soil environment system and markedly improving crop yield is the application of fertilizers [4]. Specifically, fertilization can affect microbial community composition through changes in soil properties [5], such as soil pH, total nitrogen (TN), total phosphorus (TP), and soil organic matter (SOM). Organic fertilizers can promote the diversity and activity of soil microorganisms. However, compared with organic fertilizers, chemical fertilizers are generally less effective. Different fertilization methods lead to differences in soil nutrients, pH, and microbial species [6]. Furthermore, many fertilization experiments showed that application of fertilizer had a significant influence on soil bacterial diversity and community structure and led to changes in the major bacteria in the soil [3,7–9].

Soil enzymes are produced by soil microorganisms and plant roots, which play an important role in agriculture, especially on the decomposition of organic matter and nutrient cycling [10–12]. Soil fertilization is an important factor affecting soil enzyme activity. Soil fertility is an important indicator of good crop yield, and urease plays an important role in soil quality [13]. The level of soil urease activity represents the abundance of soil nitrogen and is closely related soil nitrogen conversion, such that the activity of soil urease is often used to characterize soil nitrogen status [14]. It has been proven that using single-enzyme activity to measure soil fertility and plant yield is inaccurate [15]. Soil sucrase activity reflects the intensity of soil respiration and carbon conversion, and has been widely used to characterize the intensity and direction of the soil carbon cycle and biochemical activity [16]. Changes in catalase activity can sensitively reflect whether external environmental conditions have caused stress to plant cells, and can be used as an important indicator of excessive fertilization [17]. The level of acid phosphatase activity is closely related to the abundance of phosphorus in plants, and adequate soil phosphorus supply is an important guarantee for the normal growth of plants [18].

Previous studies mainly focused on the static effects of fertilization on soil microorganisms and enzyme activities. However, there are few studies on the effects of fertilization, especially under the condition of nitrogen reduction, on the dynamic changes of soil microorganisms and enzyme activities. In addition, most previous studies under the condition of nitrogen reduction fertilization focused on the response of soil microbial biomass and community composition, but little is known about the impact on diversity. Therefore, this study explored the effects of different nitrogen reduction fertilization on the changes of soil enzyme activities at different growth stages of rice crops, and their relationship with microorganisms. In this study, soil samples were analyzed in combination with 16S rRNA gene high-throughput sequencing to illustrate the impact of reducing fertilization and partial organic fertilizer substitution. The purpose of this study is: (a) to verify the impact of different fertilization systems on soil properties, enzyme activity, bacterial diversity and community structure; (b) identification of taxa seriously affected by fertilization system. The information obtained could help to develop more rational fertilization in farmland to maintain efficient soil fertility and increase food production.

2. Materials and Methods

2.1. Site Description and Experimental Design

The experimental field was initiated in 2019 in the Lushen Family Farm, Hegang Village, Zhangjiaji Town, Xiangzhou District, Xiangyang City, Hubei Province, China (32°07.770′ N, 112°21.812′ E), at an altitude of 76 m. The climate type belongs to the subtropical monsoon continental climate. The area had a mean annual temperature of 16 °C, and mean annual rainfall was between 878–1251 mm. The frost-free period was 240 days, and mean monthly relative humidity was 75%. Generally, there was a southeast wind in spring and summer, and a northwest wind in autumn and winter. The topography was characterized by plain terrain, and the soil type was submerged paddy soil with medium fertility. The previous crop was planted with wheat, and there was no pollution source around the experimental area. At the start of the experiment, the topsoil (0–20 cm) had a pH of 6.24, 0.86 g/kg total N, 0.65 g/kg total P, and 4.68 g/kg organic matter content.

This experiment used rice as the research crop, and the rice variety was Yongyou 4949. The experiment began in 2019, and the dates for rice sowing and harvesting were June 12 and October 15. Four treatments were set in this study, as follows (Table 1): the control treatment was fertilization methods commonly used by farmers in the past (750 kg of formula fertilizer was applied to each hectare of paddy field before transplanting, and then 90 kg and 75 kg of urea were applied at the splitting stage and booting stage, respectively); IT + RF 10 means integrated technology (carbon-based organic fertilizer substitution, adjustment of chemical fertilizer structure, and addition of zinc silicon, and boron fertilizer) and 10% reduction of nitrogen fertilizer; IT + RF 20 means integrated technology and 20% reduction of nitrogen fertilizer; IT + RF 30 means integrated

technology and 30% reduction of nitrogen fertilizer. The planting areas of the control, IT + RF 10, IT + RF 20, and IT + RF 30 groups were 0.13, 0.07, 0.07, and 0.07 hm^2, respectively. The formula fertilizer ($N:P_2O_5:K_2O$ = 20:11:14) used in the experiment was purchased from Hubei Swater Ecological Agriculture Development Co., Ltd., Xiangyang, China. In the three treatments of nitrogen fertilizer reduction, the ratio of $N:P_2O_5:K_2O$ was 22:10:15, and 1500 of kg carbon-based organic fertilizer, 6 kg of large-grained zinc, 120 kg of large-grained silicon, and 6 kg of large-grained boron were added per hectare. The urea, superphosphate, and potassium chloride fertilizer were purchased from Henan Jinmei Tianqing Coal Chemical Co., Ltd., Henan, China, Xiangyang Zedong Chemical Co., Ltd., Xiangyang, China and Sinochem Chemical Fertilizer Co., Ltd., Beijing, China respectively. The carbon-based organic fertilizer (organic matter $\geq$ 40% and effective viable bacterial count $\geq$ 20 million g^{-1}) used in this study was purchased from Wuhan Heyuan Green Biology Co., Ltd., Wuhan, China. The fertilizer application of the four treatments was manual weighing followed by mechanized applicator.

Table 1. Fertilizer application rate (kg/ hm^2).

Treatment	Urea (N: 46%)	Superphosphate (P_2O_5: 12%)	Potassium Chloride (K_2O: 60%)	Carbon-Based Organic Fertilizer	Zinc	Silicon	Boron	Formula Fertilizer ($N:P_2O_5:K_2O$ = 20:11:14)
Control	165							750
IT + RF 10	438	780	225	1500	6	120	6	
IT + RF 20	393	690	204	1500	6	120	6	
IT + RF 30	348	600	174	1500	6	120	6	

Note: IT means integrated technology; RF 10, RF 20, and RF 30 mean 10%, 20%, and 30% reduction of nitrogen fertilizer, respectively.

2.2. Soil Sample Collection and Analysis

In this study, soil samples were taken in two phases, the rice booting stage and the harvest period, in August and October 2019, respectively. Soil samples (0–20 cm) were randomly taken from each plot, and five soil cores from each treatment were combined. Rhizosphere soil samples were collected by shaking the root in a sterile plastic bag [19]. Fresh soil samples were air-dried after removing crop residues, stones, and other debris in the laboratory, and then homogenized by sieving through a 0.841 mm mesh sieve. Part of the collected soil samples were air-dried to determine soil physicochemical properties; the others were stored at −80 °C for DNA extraction or 4 °C for enzymatic activity analysis, respectively.

2.3. Analysis of Soil Physicochemical Properties and Enzyme Activities

Soil pH was measured in a volume ratio of 1:2.5 (soil:water) with a glass electrode. Total nitrogen (TN) was analyzed using the Kjeldahl digestion procedure. Total phosphorus (TP) was measured by the sulfuric acid–perchloric acid digestion method; soil organic matter (SOM) was measured by the potassium dichromate external heating method [3]. The urease, sucrase, and acid phosphatase enzymes activities were determined using a spectrophotometer (L6S, INESA, Shanghai, China). Urease was measured colorimetrically using the indophenol blue method; sucrase was determined by the 3,5-dinitrosalicylic acid colorimetry method; acid phosphatase was determined through the disodium phenyl phosphate colorimetric method; and catalase enzyme activity was measured using the $KMnO_4$ titrimetric method [3].

2.4. 16S rDNA Bacterial Sequencing Process and Data Analysis Processing

Microbial community DNA was extracted using a NucleoSpin Soil Kit (Macherey-Nagel, Germany) following the manufacturer's instructions. DNA was quantified with a Qubit Fluorometer by using a Qubit dsDNA BR Assay kit (Invitrogen Co., Ltd., Carlsbad, CA, USA), and the quality was checked by running aliquot on 1% agarose gel. Variable regions V4 of the bacterial 16S rRNA gene were amplified with degenerate PCR primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′).

Both forward and reverse primers were tagged with Illumina adapter, pad, and linker sequences. PCR enrichment was performed in a 50 µL reaction containing 30 ng template, fusion PCR primer, and PCR master mix. PCR cycling conditions were as follows: 95 °C for 3 min, 30 cycles of 95 °C for 45 s, 56 °C for 45 s, 72 °C for 45 s, and a final extension for 10 min at 72 °C. The PCR products were purified using Agencourt AMPure XP beads and eluted in Elution buffer. Libraries were qualified by the Agilent Technologies 2100 bioanalyzer. The validated libraries were used for sequencing on the Illumina HiSeq 2500 platform (BGI, Shenzhen, China) following the standard pipelines of Illumina and generating 2 × 250 bp paired-end reads.

Then, the operational taxonomic units (OTUs) were analyzed; alpha diversity was calculated by Mothur (version 1.31.2) and beta diversity metrics were calculated using QIIME (version 1.80) and the R base package (version 3.2.1). Additionally, we estimated six indices, including observed species, Chao1 richness estimator (Chao1), Abundance-based Coverage Estimator (ACE), and the Shannon, Simpson, and good-coverage indices, for alpha diversity. Here, the larger the first five and the smaller the fifth index indicated higher abundance of a species in the sample. The higher the good-coverage value, the lower the probability that the sequence in the sample is not detected. The observed species and ACE indices estimated microbial community richness; larger values indicated higher community richness. The Shannon and Simpson indices estimated microbial community diversity; higher values indicated higher community diversity.

2.5. Statistical Analysis of Sequencing Data

Differences between different treatments were estimated using one-way and two-way analysis of variance (ANOVA). Means of different treatments were compared using Duncan's test (SPSS Statistics 26).

3. Results

3.1. Soil Physicochemical Properties

Soil pH significantly increased compared with control remedy fertilizer application in treatments of IT + RF 10 and IT + RF 30 ($p < 0.05$, Table 2). Among the four treatments, SOM, soil TN, and TP of the IT + RF 20 treatment revealed the highest values ($p < 0.05$), which were 5.05, 1.06, and 0.73, respectively. In the three treatments of nitrogen fertilizer reduction, SOM, soil pH, and TN of the IT + RF 10 treatment significantly decreased compared to the IT + RF 20 and IT + RF 30 treatments ($p < 0.05$).

Table 2. Soil physicochemical properties in different fertilizer treatment.

Treatment	Soil pH	SOM (g/kg)	TN (g/kg)	TP (g/kg)
Control	6.37 ± 0.02 a	4.82 ± 0.03 c	0.96 ± 0.05 b	0.63 ± 0.01 c
IT + RF 10	6.14 ± 0.03 c	4.85 ± 0.05 c	0.77 ± 0.09 c	0.66 ± 0.01 b
IT + RF 20	6.34 ± 0.03 a	5.05 ± 0.02 a	1.06 ± 0.04 a	0.73 ± 0.01 a
IT + RF 30	6.24 ± 0.02 b	4.97 ± 0.01 b	0.97 ± 0.02 b	0.67 ± 0.01 b

Note: SOM means soil organic matter; TN means total nitrogen; TP means total phosphorus. The data represent the average of three replicates ± standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among the different treatments.

3.2. Soil Enzyme Activities and Grain Yield

3.2.1. Different Fertilization Treatments' Soil Enzyme Activities

The trend in soil enzyme activities in the four treatments differed with the growth period of the rice (Figure 1). In the booting stage, there was a significant difference in soil urease activity among the four treatments ($p < 0.05$), and the urease activity of the control and IT + RF 30 treatments were lower than that of IT + RF 10 and IT + RF 20. Compared with the control, the urease activity of the IT + RF 10 and IT + RF 20 treatments increased 49.09% and 35.82%, respectively ($p < 0.05$). With nitrogen reduction of 10% (IT + RF 10) to 30% (IT + RF 30), the soil urease, acid phosphatase, and sucrase activity significantly decreased

($p < 0.05$). However, the soil catalase activity of the IT + RF 10 and IT + RF 30 treatments significantly increased compared to the control and IT + RF 20 treatments. During the harvest period, the soil acid phosphatase activity of IT + RF 10 was significantly higher than those observed for the other three treatments ($p < 0.05$), but there was no significant change in the sucrase and catalase activities. Concurrently, under the treatment of IT + RF10, soil urease activity was higher than the control ($p < 0.05$).

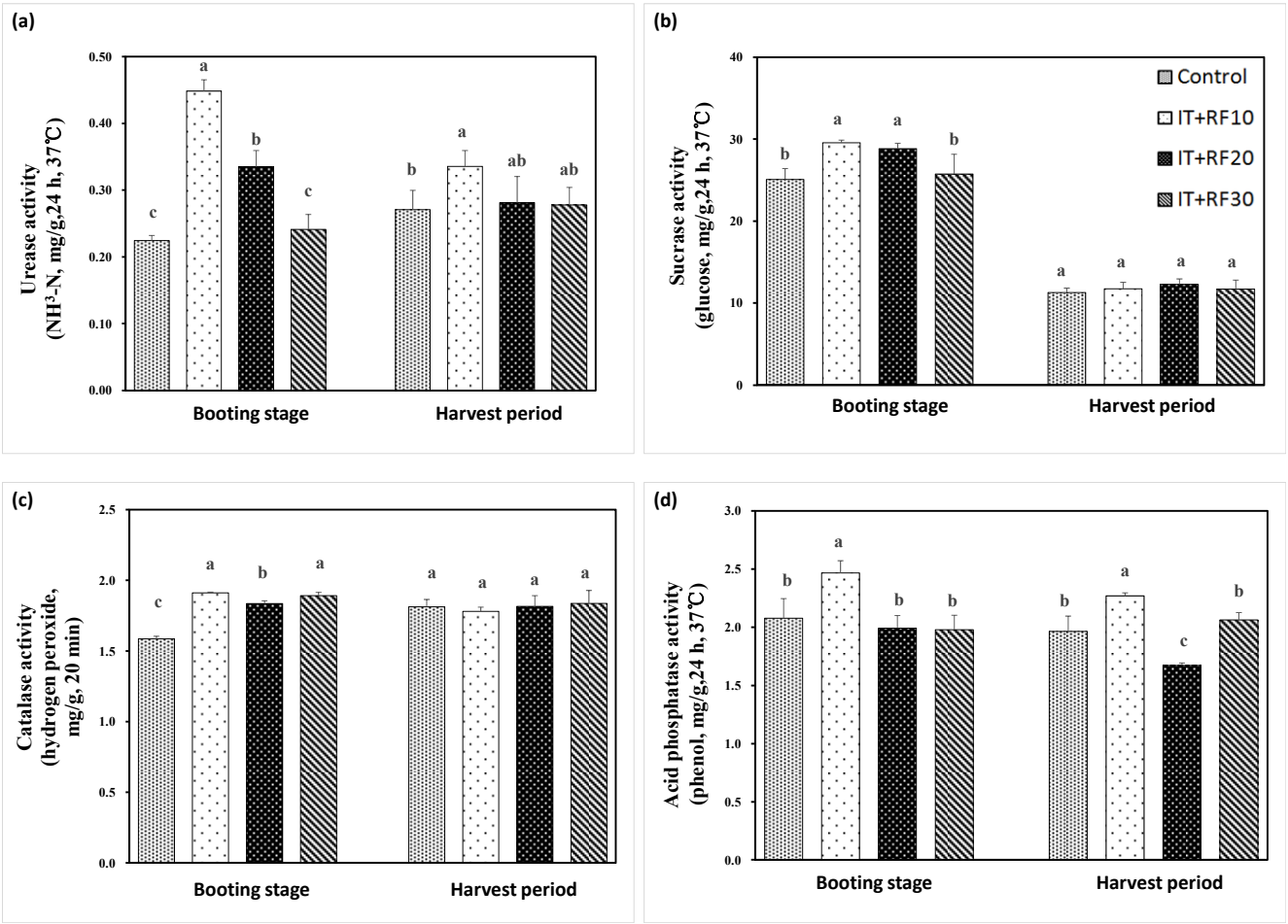

Figure 1. Soil enzyme activities under the different fertilization treatments: (**a**) urease, (**b**) sucrase, (**c**) catalase; (**d**) acid phosphatase; different lowercase letters indicate a significant difference in the same enzyme among the different treatments (n = 3, $p < 0.05$).

3.2.2. Different Fertilization Treatments' Grain Yield

In order to understand the effect of fertilization on rice yield, yields of the four different treatments were measured. The mean rice yield of the IT + RF10, control, and IT + RF20 treatments significantly increased by 10.4%, 8.2%, and 7.6% compared to the IT + RF30 treatment, respectively ($p < 0.05$, Figure 2). When the reduction rate of nitrogen fertilizer application reached 30%, rice yield was the lowest ($p < 0.05$).

3.3. Soil Microbial Diversity in Different Fertilization Treatments

3.3.1. Analysis of the Alpha Diversity of the Soil Bacterial Community

In the booting stage, observed richness (estimated as the observed OTU numbers), abundance (Chao1 and ACE index), diversity indices (Shannon and Simpson) and coverage showed no significant difference between the four treatments (Table 3). However, in the harvest period, the observed richness and diversity index (Shannon) of the IT + RF 10 treatment were lower than the control ($p < 0.05$). By comparing different growth stages, the

results showed that the observed richness and abundance (Chao1 and ACE index) in the booting stage were higher than in harvest period ($p < 0.05$).

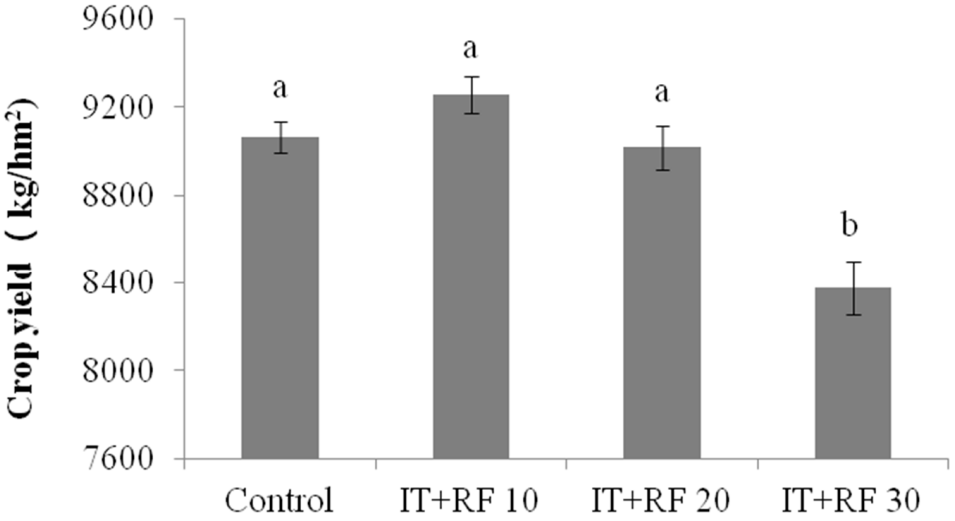

Figure 2. Crop yield of rice under different treatments. IT means integrated technology; RF 10, RF 20, and RF 30 mean 10%, 20%, and 30% reduction of nitrogen fertilizer, respectively. Different lowercase letters indicate a significant difference in the yield among the different treatments (n = 3, $p < 0.05$).

Table 3. Diversity of the microbial community among different fertilization regimes.

Period	Treatment	Observed Richness	Chao1	ACE Index	Shannon Index	Simpson Index	Coverage
Booting stage	Control	3455 ± 147 a	4331 ± 154 a	4331 ± 154 a	6.71 ± 0.15 a	0.004 ± 0.002 b	0.976 ± 0.001 bc
	IT + RF 10	3530 ± 137 a	4409 ± 103 a	4409 ± 103 a	6.73 ± 0.059 a	0.004 ± 0.000 b	0.975 ± 0.002 c
	IT + RF 20	3413 ± 91 a	4329 ± 102 a	4329 ± 102 a	6.81 ± 0.03 a	0.003 ± 0.000 b	0.976 ± 0 bc
	IT + RF 30	3459 ± 75 a	4378 ± 73 a	4378 ± 73 a	6.72 ± 0.01 a	0.003 ± 0.000 b	0.975 ± 0 c
Harvest period	Control	2898 ± 149 b	3670 ± 127 b	3670 ± 127 b	6.26 ± 0.21 ab	0.011 ± 0.003 a	0.98 ± 0.001 a
	IT + RF 10	2708 ± 269 c	3695 ± 173 b	3695 ± 173 b	5.43 ± 1.28 b	0.060 ± 0.076 a	0.979 ± 0 ab
	IT + RF 20	2885 ± 130 bc	3771 ± 283 b	3771 ± 283 b	6.34 ± 0.06 ab	0.007 ± 0.001 a	0.979 ± 0.002 ab
	IT + RF 30	3092 ± 76 b	3926 ± 46 b	3926 ± 46 b	6.56 ± 0.14 a	0.005 ± 0.001 a	0.979 ± 0.002 a

Note: IT means integrated technology; RF 10, RF 20, and RF 30 mean 10%, 20%, and 30% reduction of nitrogen fertilizer, respectively. Data represent the average of three replicates ± standard deviations. Same lowercase letters indicate no significant differences ($p < 0.05$) among the different treatments.

3.3.2. Composition of the Soil Bacterial Community

The top 10 species with the highest abundance at the phylum classification level were selected to generate a relative abundance histogram (Figure 3), and the same four groups dominated (*Proteobacteria*, *Acidobacteria*, *Chloroflexi* and *Verrucomicrobia*) in both the booting stage and harvest period. In the booting stage, as shown in the figure, across the four treatments the proportion of *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, and *Verrucomicrobia* ranged from 30.6–35.7%, 15.1–19.2%, 10.0–12.3%, and 8.77–9.76%, respectively. During the harvest period, the proportion of *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, and *Verrucomicrobia* ranged from 30.2–45.8%, 14.5–20.7%, 5.57–13.5%, and 8.10–16.4%, respectively.

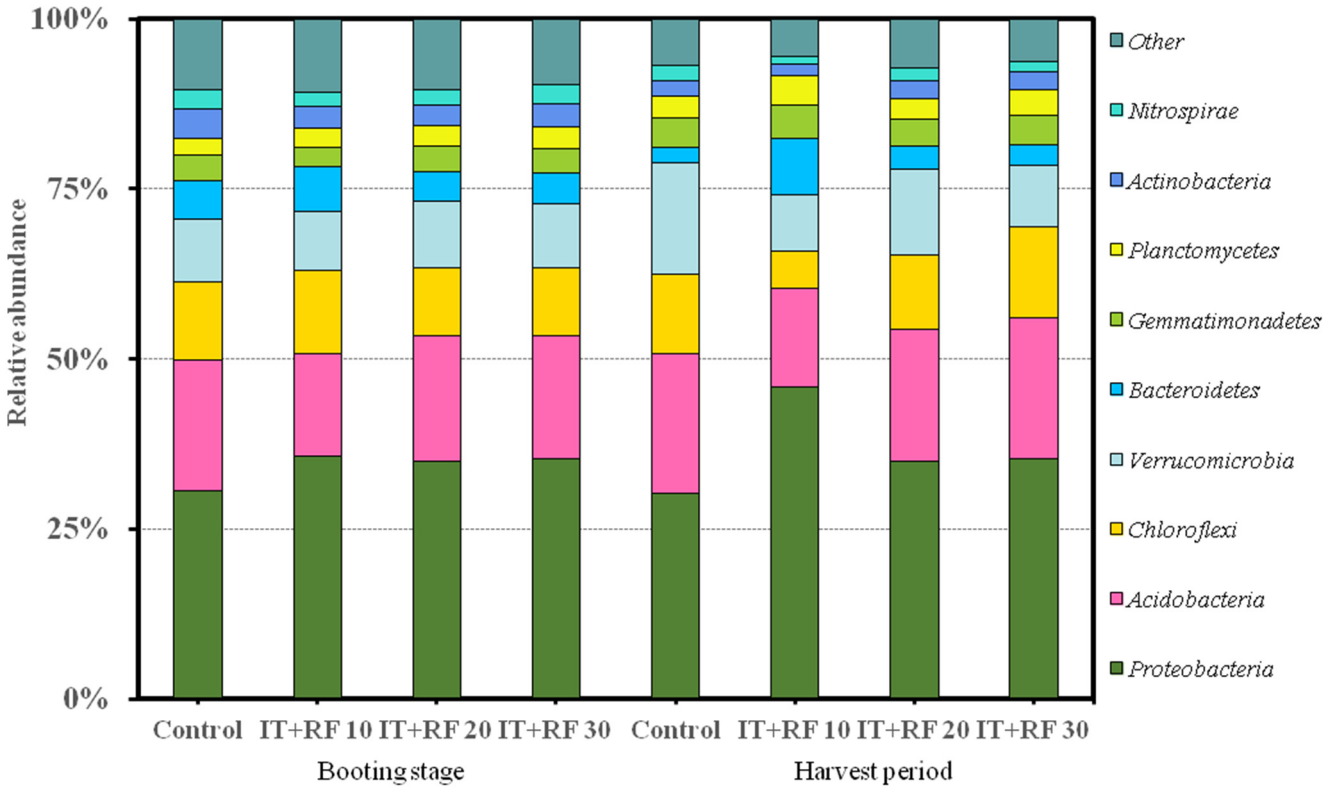

Figure 3. Comparison of the bacterial communities at the phylum level of different treatments. Sequences that could not be classified, or species abundance of less than 0.5% in any known group, were labelled 'Other'. IT means integrated technology; RF 10, RF 20, and RF 30 mean 10%, 20%, and 30% reduction of nitrogen fertilizer, respectively.

4. Discussion

4.1. Effects of Reduced Fertilization on Rice Yield and Soil Properties

The yield of different fertilization treatments showed significant differences, which may be due to an insufficient available nitrogen supply during the late growth stage of rice caused by the 30% reduction in nitrogenous fertilizer; this would lead to premature senescence of leaves, weakening the photosynthesis of crops and ultimately affecting the yield. In addition, organic fertilizer can provide a large amount of energy for soil microorganisms after being applied to the soil, which can coordinate the supply of soil nitrogen, thus improving the nutrient utilization rate.

The reduction of nitrogen fertilizer treatments in the present study significantly changed the bulk soil pH in the rice cropping system. An acidifying effect of urea and ammonia fertilizers in agricultural fields is well known [20]. The acidity generated by fertilization is mainly a result of nitrification, which produces concomitantly to the formation of nitrite and nitrate protons [21]. Consistent with previous results [22,23], the soil TP and organic matter increased considerably in the 20% and 30% reduction of nitrogenous fertilizer treatments, compared to the other treatments. Generally, carbon-based organic fertilizer provides an important source of organic C for soil microorganisms in agro-ecosystems [24], which can significantly increase the soil organic matter contents [25]. This has been widely recommended as an environmentally-friendly practice to balance the C loss owing to mineralization and improve soil fertility in agricultural soil [26,27].

4.2. Effects of Reduced Fertilization on Enzyme Activities

Enzymes are important for nutrient cycling in soil, and most soil enzymes are from microorganisms [28]. It has been reported that mineral fertilization may reduce the activities of C-, N- and P-related hydrolases [29–31], because of the reduction of soil heterotrophic

respiration. On the contrary, this study showed that a combination of organic fertilizer and mineral fertilization could stimulate most enzyme activities in the rice planting system. This may be related to the increase of soil organic carbon and microbial biomass after fertilization [21]. Previous studies also showed that enzyme activity was stimulated after nitrogen fertilizer and straw were returned to the field [32,33]. On the other hand, soil enzyme activity was inhibited after conventional nitrogen fertilizer [11]. However, the increase of enzyme activity seems to be related to the fertilizer form and application rate. In this study, the highest urease activity was observed in IT + RF 10, followed by IT + RF 20 and IT + RF 30, in both stages, and the same trend was seen in sucrase and acid phosphatase in the booting stage, indicating that there was a relationship between enzyme activity and application rate. This phenomenon showed that organic fertilization can stimulate soil enzyme activity more effectively. Compared with inorganic fertilizers, organic fertilizers have a stronger impact on soil enzyme activity [34–36]. The application of organic fertilizer can increase the content of soil nutrients and change the soil microbial community, thus enriching the source of soil enzymes [37–39]. Organic fertilizer can improve soil factors, and studies have shown that the environmental factors of soil, such as pH, organic matter, TN, and TP, are related to the composition and diversity of the soil microbial communities [3], which also affect soil enzyme activity [20]. The results of this study showed that the soil urease and phosphatase activities of the IT + RF 10 treatment were significantly higher than those of the control. In the booting stage, the urease and sucrase activities of IT + RF20, and the catalase activities of IT + RF20 and IT + RF30, were higher than those of the control. This means that organic fertilizer can significantly improve enzyme activities related to the carbon, nitrogen, and phosphorus cycles in soil [40]. The reason may be that organic fertilizer can significantly improve the physical and chemical properties of soil, promote crop root system growth, and improve soil enzyme activities. In addition, organic matter can provide a large amount of carbon source for soil microorganisms and cause them to produce more extracellular enzymes [41].

4.3. Effects of Reduced Fertilization on Soil Bacterial Diversity and Community Composition

Soil microorganisms play critical roles in soil function and productivity through their involvement in nutrient cycling and organic matter turnover [3]. Deep 16S rRNA gene sequencing was used to investigate the bacterial community structure in paddy soil. Across all treatments, communities were dominated by *Proteobacteria*, *Acidobacteria*, *Chloroflexi* and *Verrucomicrobia*, which corresponds at the phylum level to the results of previous studies in similar soils [42]. In this study, the IT + RF 10 treatment showed the largest observed richness, ACE index, Shannon index, and Simpson index, which means that the soil bacteria richness of this treatment method was the highest.

In all treatments of paddy soil, the differences in bacterial community composition at the phyla level was small. Similar results have been reported previously, and statistically significant differences in bacterial communities could not be detected in different treatment schemes [42–44]. On the contrary, some studies reported significant changes in soil bacterial diversity and community composition after long-term fertilization [45]. This is partly due to differences in experimental systems, management practices, and even sequencing techniques [46]. A meta-analysis of 107 data sets based on 64 long-term trials around the world showed that fertilization has a significant impact on soil microorganisms in the agricultural system, depending on the pH value [21]. However, when nitrogen fertilizer reduces soil pH value, it will affect soil microbial biomass, activity, and community composition [21]. Consistent with these findings, the soil pH values of the control, IT + RF 10, and IT + RF 30 treatments in this study were different, and the relatively stable microbial diversity and community composition were observed to be slightly different. In addition, a recent study also emphasized the importance of seasonal change on soil microbial community composition, and showed that the fertilization effect was generally significant in June, but not so obvious in October [7]. The high temperature and precipitation in the late rice growing season in summer may cause significant changes in the soil microbial community, which

may affect the effect of fertilization treatment. The harvest date is October, which can partly explain the relatively weak effect of fertilization on microbial diversity and community structure in this study.

5. Conclusions

In summary, this study showed that a reduction in chemical fertilizer application has a certain impact on the soil pH, TN, TP, and SOM values, as well as the proportion of dominant groups of soil microorganisms, in the rice planting system. The IT + RF 10 treatment was the best way to reduce nitrogenous fertilizer application, which can reduce nutrient loss in the paddy soil while maintaining the activity of soil enzymes and the abundance of bacteria. The application of organic fertilizer partially replaces chemical fertilizer, which not only effectively stimulates soil enzyme activity, but also enriches bacterial groups that may participate in complex organic matter decomposition and soil nutrient mobilization. Therefore, under the condition of not over-applying chemical fertilizers, the best combination of reducing chemical fertilizers and organic fertilizers can effectively improve the availability of soil nutrients.

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