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Soil microbial community composition in a paddy field with different fertilization managements

Limin Wang ^{1,2}, Dongfeng Huang ^{1,2, *}

¹ *Soil and Fertilizer Institute, Fujian Academy of Agricultural Sciences, Fuzhou 350013, P.R.*

China

² *Fujian Key Laboratory of Agro - products Quality & Safety, Fujian Academy of Agricultural Sciences, Fuzhou 350000, P.R. China*

*Corresponding author: Dongfeng Huang

Soil and Fertilizer Institute,

Fujian Academy of Agricultural Sciences,

No. 100, Pudang Village, Xindian Town, Fuzhou City, Fujian Province, China.

Zip code: 350013.

E-mail: hdf_2169395@126.com

Tel:+86-591-87572673

Fax:+86-591-87572514

ABSTRACT

Microbes play vital roles in soil quality; however, it remains poorly understood about their response to N (nitrogen) and P (phosphorus) fertilization in acid paddy soils of subtropical China. Here, a 10-year field experiment was conducted to evaluate the effects of different fertilization treatments on microbial communities by Illumina Miseq sequencing. Results showed that different fertilization treatments did not exert a significant effect on microbial alpha diversity, but altered soil properties and thus affected microbial community composition. The microbial communities in the T₁ (optimized N and P fertilizer)- and T₂ (excessive N fertilizer)- treated soils differed from those in the T₀ (no N and P fertilizer)- and T₃ (excessive P fertilizer)- treated soils. In addition, the bacterial phyla *Proteobacteria*, *Chloroflexi* and *Acidobacteria*, and the fungal phyla *Ascomycota* and *Basidiomycota* dominated all fertilized treatments. Furthermore, soil TK (total potassium) concentration was the most important factor in driving the variation in bacterial community structure under different fertilization regimes, while the major factors shaping fungal community structure were soil TN and NO₃⁻-N (nitrate N). These findings indicated that optimization of N and P application rates might result in variations in the soil properties which changed the microbial community structure in the present study.

Keywords: Illumina Miseq, nitrogen and phosphorus fertilization, community structure, soil property

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the main staple crops and feeds over 65% of the world's population (Saikrishna et al. 2018). Because the population is steadily increasing, rice production must increase by 1% annually (Chen et al. 2018). Hence, an increasing demand for rice plant productivity is one of the main challenges in the 21st century. The application of N (nitrogen) and P (phosphorus) fertilizers is an indispensable agricultural practice to improve rice production to meet the growing demand for rice (Oshunsanya et al. 2019). However, the excessive use of chemical fertilizers could cause soil quality deterioration and surface water eutrophication (Lou et al. 2017; Yi et al. 2018). More than half of the applied N and P fertilizers would be lost from the fertile farmland where N was lost by forming N_2 , trace gases, and nitrates, and P was lost by forming metal complexes in the soil (Lentz and Lehrsche 2018). To overcome these constraints, the need to reduce anthropogenic inputs of N and P to rice ecosystems has been widely recognized. The optimization of fertilizer management is an effective measure to produce high and stable grain yields by supplying an appropriate amount of N and P to rice plants with no deleterious effects during the growing seasons.

The optimization of N and P fertilizer rate simultaneously played a positive role in regulating microbial community structure by changing soil properties, and then exerted a positive impact on soil quality and rice productivity in field conditions (Fang et al. 2019; Huang et al. 2019). Soil microorganism, a key driver of soil biogeochemical processes, played a vital role in maintaining soil productivity. Related studies have suggested that ammonia-oxidizing archaea (AOA) played an important role in the ammonia oxidation in a paddy soil (Fang et al. 2019). In contrast, Wang et al. (2017a) found that ammonia-oxidizing bacteria (AOB) might be responsible for the major source of

nitrite N (NO_2^- - N) in the phreatic zone. Additionally, applications of plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi enhanced the uptake of N and P elements, which was beneficial for obtaining high rice yield (Nath et al. 2018). In general, the knowledge of how soil microbial communities respond to N and P fertilizer application is important for selecting suitable fertilizer management strategies to provide subtropical rice paddy fields with beneficial ecosystem services. However, the effects of N and/or P fertilizer application on microbial diversity and composition were currently inconsistent among various studies. Yao et al. (2021) reported that soil bacterial community richness increased with N application rate in a coastal salt-affected agroecosystem. On the contrary, Wang et al. (2018) found that the N application reduced soil bacterial richness, while the external P input altered community composition in an old-growth N-rich tropical forest. In addition, He et al. (2016) showed that soil fungal community structure was more sensitive to P than N application in the alpine meadow on the Qinghai-Tibetan Plateau. These inconsistent results might be due to different ecosystem types, N or P fertilizer forms and doses. Moreover, microbial community composition and diversity were reportedly altered over a wide range of soil factors associated with different fertilizer managements (Fang et al. 2019; Yi et al. 2018).

Microbes are the most abundant and diverse organisms in soils. High-throughput sequencing (HTS) could provide significant insights into an extremely high microbial diversity in soils, due to its high resolution. Hence, the HTS technique is increasingly used for soil microbes (Wang et al. 2019; Xu et al. 2018). Previous studies have mostly focused on the impacts of N and/or P additions on soil microbial community (Dai et al. 2018; He et al. 2016; Wang et al. 2018), but little information is available on the combined effects of optimizing reduction in N and P fertilizers on soil microbial community in subtropical paddy soils. Therefore, the response of soil microbial communities to N and

P fertilizer reduction in paddy fields of subtropical China is poorly understood. In this study, we conducted a 10-year fertilization experiment in a subtropical paddy field to test the hypothesis that optimized N and P fertilization would lead to a different soil microbial response compared to excess N and P application.

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MATERIALS AND METHODS

Site description

Field trial was initiated in 2008 and cropped by double-cropping rice (*Oryza sativa* L.) annually at Baisha Experimental Station, Fuzhou, Fujian Province, China (26°13'31"N, 119°04'10"E). The early and late cultivars of rice are conventional rice varieties 78-30 and 428, respectively, from south China. The site is characterized by a subtropical monsoonal climate with a mean annual temperature and precipitation of 19.5 °C and 1 350 mm, respectively. The soil in the area is classified as a typic Hapli-Stagnic Anthrosol (USDA soil system) with a pH (1:2.5) 6.19, 14.16 g kg⁻¹ SOM (soil organic matter), 0.66 g kg⁻¹ TN (total N), 0.30 g kg⁻¹ TP, 3.8 mg kg⁻¹ NO₃⁻-N (nitrate N), 12 mg kg⁻¹ ammonium N (NH₄⁺-N), 3.358 and 0.83 mg kg⁻¹ of available P (AP) and potassium (AK), respectively, at the beginning of experimentation.

Experimental treatments

Twelve plots (4 m × 5 m per plot) were established for fertilization trials. The trials were laid down in a randomized complete block design. The treatments included control (T₀, no N and P fertilizer), optimized N and P fertilizer (T₁, based on fertilizer recommendation from local agriculture committee), excessive N fertilizer (T₂), and excessive P fertilizer (T₃) with three replicates. The excess amounts of N or P applied to the paddy soils were calculated according to the recommended fertilizer rates for rice grown in yellow-clayed paddy fields in southern China (Chen 2000). The fertilizer practices were described in Table 1. The fertilizers in this experiment were urea (46% N), superphosphate (5% P), potassium chloride (50% K), and chemical compound fertilizer (15% N, 7% P, 12% K), respectively. N was applied at graded levels as per the treatments in two splits i.e., two thirds at basal and one third at

tillering, and K was one third at basal and two thirds at tillering. The total amount of P fertilizers were applied as a basal dose (Table 1). Annual fertilizer application rates were the same since 2008. In order to prevent the exchange of water and nutrients between adjacent plots, a concrete cement border (40 cm length $\times$ 30 cm width $\times$ 20 cm height) was built. The early rice was transplanted with a 20.0 cm $\times$ 23.0 cm hill spacing on 21 April and harvested on 25 July 2018. The late rice was transplanted with the same hill spacing on 30 July and harvested on 1 December 2018.

Soil and plant physicochemical analysis

The rice straw and grain were sampled and their yields were measured at harvest from each plot, separately (rice grain weights were adjusted to 13.5% moisture content). A soil sample consisted of five soil cores. The soil sample was collected at 0-20 cm soil layer in each plot after late rice harvest, and then homogenized as a composite sample. These samples were transported immediately on ice to the laboratory. One subsample was stored at - 80 °C for molecular analysis, and the remaining soil was air-dried and passed through 2 mm sieve before analysis. Soil moisture was calculated as the difference between oven-dry (24 h at 105 °C) and fresh weight. Soil pH was measured with a pH meter on 1:2.5 (w/v) soil-water suspensions. Soil organic C (SOC) was determined by the $K_2Cr_2O_7$ oxidation-reduction titration technique. The TN content was measured by the potassium persulfate oxidation ultraviolet photometric method. Both NH_4^+ -N and NO_3^- -N in 2 M KCl soil extracts (1:10 soil/extract (wt:vol)) were determined with UV spectrophotometry (Bao 2000). TP was analyzed by the alkaline fusion molybdenum-antimony colorimetric method, and Olsen P was determined by using the molybdate blue colorimetric method by extracting the samples with 0.5 M $NaHCO_3$ (Olsen and Sommers 1982). The TK and AK contents were determined by flame photometry (Bao 2000).

DNA extraction and PCR amplification

Soil DNA was extracted from 0.5 g fresh soil by using an E.Z.N.A Soil DNA Kit (Omega Bio-tek, Norcross, Georgia, USA), according to the manufacturer manual. The purity and concentration of the extracted DNA was determined by a NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The V3 - V4 region of the bacterial 16S rRNA genes was amplified by PCR (95 °C for 3 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s and a final extension at 72 °C for 10 min). The amplification of the 16S rRNA genes was performed by using the forward primer 338F (5'-barcode- ACTCCTACGGGAGGCAGCA -3') and the reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'), where a barcode was an unique eight-base sequence for each sample. Meanwhile, the V4-V5 region in the fungal 18S ribosomal RNA genes was amplified by PCR (95 °C for 3 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 10 min) using primers SSU0817F 5'-barcode-TTAGCATGGAATAATRAATAGGA)-3' and 1196R 5'-TCTGGACCTGGTGAGTTTCC-3', where a barcode was an unique eight-base sequence for each sample. PCR reactions were performed in triplicate. The PCR mixture (20 µL) contained 4 µL 5 × FastPfu Buffer, 2 µL 2.5 mmol L⁻¹ dNTPs, 0.8 µL each primer (5 µmol L⁻¹), 0.4 µL FastPfu Polymerase, and 10 ng template DNA.

Illumina MiSeq Sequencing

The amplified DNA was subjected to horizontal electrophoresis on 2% agarose and purified with an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, California, USA) according to the manufacturer's instructions and quantified by using QuantiFluo-ST (Promega, Madison, Wisconsin,

USA). The purified amplicons were pooled in equimolar concentrations and paired-end sequenced (2×250) on an Illumina MiSeq platform according to the standard protocols. The raw reads were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database with accession number SRP293735.

Illumina Data Analysis

Raw fastq files were demultiplexed, quality-filtered, and analysed by Quantitative Insights Into Microbial Ecology (QIIME) 1.17 (Caporaso et al. 2010). These sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity by using the UPARSE pipeline (Edgar 2013). Chimeric sequences were identified and removed by using UCHIME. The taxonomy of 16S and 18S rRNA gene sequences was analyzed by RDP Classifier (<http://rdp.cme.msu.edu/>) against the Silva rRNA database by using a confidence threshold of 70% (Caporaso et al. 2012). Furthermore, rarefaction curves and other OTUs-based analyses such as the abundance-based coverage estimators (ACE) and Chao 1, Shannon-Wiener index (H'), and Simpson's index (D) were calculated by using the mothur software package. Chao 1 and ACE were used to estimate microbial richness based on sequence dissimilarity. The microbial alpha diversity within each sample was estimated by H' and D (Lim et al. 2010).

Data analysis

Statistical analyses were performed by using SAS version 8.02 (SAS Institute Inc., Carey, North Carolina, USA). All values were expressed as means $\pm$ SD ($n=3$). The one-way analysis of variance and the Duncan multiple-range test were used to assess the significant effects of N and P fertilization on microbial diversity, edaphic characteristics, and rice yields. In addition, the number of shared and

unique OTUs among the treatments was described by a Venn diagram. To compare the microbial phyla among different fertilization treatments, a heatmap analysis was performed, and the result was plotted in Rstudio by using the pheatmap package. Furthermore, principal coordinates analysis (PCoA) and analysis of similarity (ANOSIM) with Bray-Curtis distance, and partial least squares discriminant analysis (PLS - DA) were used to evaluate beta diversity, in order to explore similarities and differences in microbial community structure. Meanwhile, a heatmap of correlations between the relative abundances of microbial phyla and edaphic characteristics (e.g., pH, SOC, and TN) was tested by the Canoco software for Windows Version 4.5. In addition, soil factors were selected by the functions of envfit (permu = 999) and variance inflation factor (vif).cca, and these factors with vif > 10 were removed from the following analysis. The vif values of SOC, TP, and AK were higher than 10 and removed. To determine soil variables related to microbial community structures, the distance - based redundancy analysis (db - RDA) was performed by R software (version 3.3.1).

RESULTS

Rice yields and soil fertility

Rice grain yields in double-rice cropping season during the 2008-2018 period in the T_1 -, T_2 -, and T_3 - treatments were increased by 1.69, 1.77, and 1.69 times, stover yields in double-rice cropping season by 0.92, 1.03, and 1.00 times compared with those in the T_0 treatment, respectively (Fig. 1A, B). However, there were no significant differences in the average rice grain and stover yields in 2008-2018 under the T_1 -, T_2 -, and T_3 - treatments (Fig. 1A, B). Therefore, the optimizing N and P fertilizer reduction could maintain high stover and grain yields in double-cropping rice in the studied area. Meanwhile, soil available nutrients (i.e., NH_4^+ -N, AK and Olsen P) significantly ($P < 0.05$) increased in the T_1 , T_2 , and T_3 treatments compared with those in the T_0 treatment (Table 2). On the contrary, the NO_3^- -N content significantly ($P < 0.05$) decreased in the T_1 and T_3 treatments compared to that in the T_0 treatment (Table 2). In addition, the pH value ranged from 5.94 ± 0.08 to 6.27 ± 0.18 , indicating that four paddy soils were acidic (Table 2).

Microbial diversity

High query coverage (>98.0%) suggested that this study captured the dominant OTUs of microbia in each soil sample (Table 3). Moreover, all of the rarefaction curves of bacterial 16S rRNA and fungal 18S rRNA sequences in soil samples reached saturation, suggesting that the number of sequence reads was sufficient to represent most of sequence types (Fig. 2A, B). The numbers of 16S rRNA OTUs from bacteria at a 97 % sequence identity were 2250, 2330, 2351 and 2310, as well as 270, 264, 265 and 292 for fungal OTUs in soil samples in the T_0 , T_1 , T_2 and T_3 treatments, respectively (Fig. 3A, B). Most bacterial OTUs (82.94%) were shared among four treated soil samples (Fig. 3A), while only 188 of 363 fungal OTUs were shared (Fig. 3B). Additionally, bacterial alpha diversity indices (ACE, Chao 1 and

Shannon-Wiener index) were significantly ($P < 0.05$) higher than those of the fungi in paddy soils under different fertilization regimes (Table 3). However, soil microbial alpha diversity was nonsignificantly different among different treatments (Table 3). No variations in the soil microbial alpha diversity among the different treatments may be explained by their response to natural mechanisms rather than by direct impacts of chemical fertilization on bacteria and fungi. Similarly, for beta diversity, there was no significant change in microbial community structure by PCoA with Bray-Curtis distance (Fig. 4A, B), irrespective of fertilization regimes. Furthermore, ANOSIM tests confirmed no significant differences in the composition of the bacterial ($R = -0.18$, $P = 0.90$) and fungal ($R = 0.13$, $P = 0.81$) communities (Fig. 4A, B). However, the replicate samples T_{01} , T_{02} , and T_{03} , the samples T_{11} , T_{12} , and T_{13} , the samples T_{21} , T_{22} , and T_{23} , and samples T_{31} , T_{32} , and T_{33} within the same treatment could not be closely clustered into a group (Fig. 4A, B), suggesting that the experimental replication in the same treatment was not good in this field trial, possibly because of spatial variation in soil microbial communities across field experimental plots. Under these circumstances, a supervised method, PLS - DA, was chosen to maximize the variance among different soil samples, while minimizing the variance within each sample (Barker and Rayens 2003). PLS - DA revealed that soil microbial structure in the T_1 treatment was more similar to that in the T_2 treatment than that in the T_3 treatment (Fig. 4C, D). A total 27.72% of the variations in the bacterial community composition could be explained by the first two principal components, and a total 21.52% of the variations in the fungal community structure by the first two principal components (Fig. 4C, D).

Microbial community composition

Proteobacteria, *Chloroflexi*, and *Acidobacteria* were the predominant bacterial phyla in paddy soils, with their relative abundances of 28.30–34.55%, 21.46–30.70% and 8.73–10.68%, respectively; the

rare phyla were *Bacteroidetes* and *Gemmatimonadetes* with low abundances (Fig. 5A). Moreover, the T_1 treatment increased the relative abundances of the bacterial phyla *Proteobacteria*, *Firmicutes*, and *Nitrospirae*, but reduced the abundance of *Chloroflexi* compared to those in other treatments (Fig. 5A). Additionally, the classified sequences from each treated soil were affiliated with the fungal phyla: *Ascomycota*, *Basidiomycota*, *Chytridiomycota* and *Mucoromycota* (Fig. 5B). *Ascomycota* and *Basidiomycota* were the two most abundant fungal phyla in all soil samples (Fig. 5B). Furthermore, the T_1 and T_2 treatments increased the relative abundance of *Chytridiomycota* compared with other two treatments (Fig. 5B).

Relationships between soil microbial community composition and edaphic factors

Soil TK ($r^2=0.6531$, $P=0.011$) was significantly correlated with bacterial community structure by using a db - RDA (Fig. 6A). Furthermore, the relative abundances of the bacterial phyla *Proteobacteria*, *Firmicutes*, and *Nitrospirae* were significantly positively correlated with soil $\text{NH}_4^+\text{-N}$ content ($P < 0.05$), while the relative abundance of *Cyanobacteria* was significantly positively related to soil TP content ($P < 0.05$) (Fig. 7A). Additionally, soil TN ($r^2 = 0.6536$, $P = 0.007$) and $\text{NO}_3^-\text{-N}$ contents ($r^2 = 0.5455$, $P = 0.034$) were significantly related to fungal community composition (Fig. 6B). The first ordination db - RDA axis (axis 1, horizontal), which was strongly correlated with the TN content, explained 15.87% of the variability in fungal community structure (Fig. 6B). The second ordination RDA axis (axis 2, vertical) was mainly related to TN and $\text{NO}_3^-\text{-N}$ contents, and explained 9.59% of the total variability in fungal community composition (Fig. 6B). Moreover, the relative abundance of *Mucoromycota* fungi was significantly positively correlated with soil TK content ($P < 0.01$), and the relative abundance of *Ascomycota* fungi was significantly positively correlated with the SOC content ($P < 0.05$) (Fig. 7B).

DISCUSSION

Long-term chemical fertilization could influence soil properties, and thus altered microbial community diversity (Xiang et al. 2020). In this study, there were no significant alterations in microbial alpha diversity among different fertilization regimes, suggesting that it remained steady after N and P fertilizer application in the paddy soil (Table 3). This finding was consistent with the previous study, which revealed that P application had no significant effect on soil bacterial alpha diversity (Wang et al. 2018). However, these results were inconsistent with these findings (Dai et al. 2018; He et al. 2016; Xu et al. 2020), which indicated that long-term chemical fertilization led to the decline of bacterial diversity. Furthermore, the reduction in microbial diversity in more concentrated chemical fertilizer treatments was greater than that in lower concentrations (Zhou et al. 2015). The different response of bacteria and fungi to the application of N and/or P fertilizers could be attributed to the great heterogeneity in soil properties, crop types, and fertilization rates (He et al. 2016; Xie et al. 2014). In addition, the bacterial alpha diversity was significantly higher than the fungal one ($P < 0.05$) in this study (Table 3), probably because they were smaller with higher mutation rate, and diverse metabolic capacities than the fungi (Güsewell and Gessner 2008; Liao et al. 2018; Liu et al. 2018). Meanwhile, soil fungal alpha diversity showed a decreasing trend in the T_3 -treated soil compared to that in the T_0 -treated soil (Table 3). This was because the soil with a lower N:P ratio under the T_3 treatment following higher P application rate, where it was generally dominated by fast-growing bacteria (Güsewell and Gessner 2008). The bacteria have particularly high demands for P because high rates of cell division require large amounts of P-rich RNA (Elser et al. 2003). Taken together, our findings indicated that different fertilizer treatments had no significant influence on microbial alpha diversity in

acid paddy soils.

Different fertilizer treatments, however, shaped different microbial communities by PLS - DA (Fig. 4A, B). This result was consistent with the finding of Fang et al. (2019), who reported that fertilization significantly altered soil microbial community composition, indicating that N and P application induced the change in microbial community structure. Moreover, the microbial communities in the paddy soils of the T₁ and T₂ treatments were clustered together and separated from the T₃ treatment (Fig. 4C, D), which implied that excessive P application affected soil microbial community structure more strongly than excessive N fertilization. Similarly, previous studies demonstrated that soil fungal community composition was more sensitive to P than N addition in both an alpine meadow and a secondary tropical forest (He et al. 2016; Wang et al. 2018). It was because the soil N:P ratio, as a key factor influencing the competitive advantage of different fungal taxa, was significantly reduced by P application alone, where non-mycorrhizal fungi were more competitive than mycorrhizal fungi (Chagnon and Bradley 2013; Liu et al. 2010).

The fertilization-induced change in microbial community structure may be caused by their responses to soil property alteration. This study indicated that the most predominant factor affecting soil bacterial community structure was TK (Fig. 6A). Likewise, Luo et al. (2017) demonstrated that SOC, TN, and TK were the key factors influencing microbial community structure. These results suggested that soil TK was a candidate to (co-) limit soil microbial community structure. The reason was that K was a major intracellular cation in all kinds of organisms including bacteria and fungi, and their growth was affected by soil TK content (Liu et al. 2016; Weed et al. 1969). On the contrary, Sun et al. (2015) suggested that soil properties (except TK) had a significant effect on soil bacterial communities. The correlations between bacterial communities with different soil properties could

depend more on the different soil types. It has been reported that bacterial community structure was associated with SOC, AK and AN contents in granite soil, but was more closely related to TP and pH in purple sandy shale soil (Sun et al. 2015). In addition, the main factors governing fungal community composition were TN and NO_3^- -N under different fertilization regimes (Fig. 6B). These results indicated that bacteria and fungi could be limited by different nutrients, respectively. It has been reported that the bacterial community was closely related to the shifts of soil P, while the fungal community was strongly associated with the changes of soil C and N (Liao et al. 2018). In addition, many previous studies suggested that microbial community structure was mainly affected by soil nutrients including SOC, TN, and NO_3^- -N contents (Cheng et al. 2017; Xiang et al. 2020). However, Liu et al. (2018) showed that soil pH rather than nutrients drove changes in microbial community composition in acidic ultisols of southern China. The reason for this inconsistency largely depended on the magnitude of soil pH change. When soil pH showed a little change, soil nutrient conditions were the main factors influencing microbial community composition; when soil acidification or alkalization was exacerbated, the influence of soil pH gradually increased, whereas the roles of other edaphic factors in determining microbial community structure were relatively small (Xun et al. 2015). In this study, the soil samples of all fertilization treatments had the narrow pH range 5.97-6.27; thus, other soil nutrients became the deterministic factors affecting microbial community composition.

The relative abundance of *Proteobacteria* was significantly positively correlated with soil NH_4^+ -N content ($P < 0.01$) (Fig. 7A). In addition, *Proteobacteria* was the overwhelmingly dominant bacterial phylum in all soil samples (Fig. 5A), because members of the phylum could utilize a wide range of complex organic molecules and survived in various and sometimes extreme habitats (Bouskill et al. 2010). Likewise, *Bacteroidetes*, as an r-strategist (Fierer et al. 2007), could be favored by high soil

NH_4^+ -N content (Fig. 7A). Additionally, *Firmicutes*, as N mineralizers during warm seasonal periods, could fix N_2 , and consequently produced significant amounts of soil NH_4^+ -N during rice plant growth (Jha and Saraf 2015; Santana et al. 2013; Wang et al. 2017b). Our results further demonstrated that soil NH_4^+ -N content was significantly positively related to the relative abundance of *Firmicutes* ($P < 0.01$) (Fig. 7A). The enrichment of *Nitrospirae*, as a facultatively anaerobic chemoautotrophic nitrite oxidizer, had positive effects on high potential denitrification rates, produced nitrous oxide, increased gaseous N loss from paddy fields, and consequently decreased soil TN content (Fang et al. 2019; Shaw et al. 2006), which agreed with our finding (Fig. 7A). Some cyanobacterial taxa could drive P cycling by accessing P pools that are not generally available to plants (Cottingham et al. 2015). Hence, the ability of *Cyanobacteria* contributed to increase soil P content with an increase in their relative abundance, which was supported by our study (Fig. 7A). Additionally, *Ascomycota*, one of the most abundant fungal phyla (16.72 - 23.07% of OTUs) (Fig. 5B), dominated the mycorrhizal networks that translocated C and N between biocrusts and plants (Sinsabaugh et al. 2011), and were significantly positively associated with SOC content ($P < 0.05$) (Fig. 7B). In conclusion, our results showed that the optimizing N and P fertilizer reduction might alter soil properties, and consequently mediate microbial community structure in the present study.

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Figure Captions

Fig. 1. Grain (A) and stover (B) yield (kg ha^{-1}) of rice as influenced by fertilization in 2008-2018. Notes: T_0 = No nitrogen and phosphorus fertilizer; T_1 = Optimized nitrogen and phosphorus fertilizer; T_2 = Excessive nitrogen fertilizer; T_3 = Excessive phosphorus fertilizer. The different lower-case letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.

Fig. 2. Bacterial (A) and fungal (B) Shannon–Wiener curves for normalized number of reads at a 97% threshold in different fertilization regimes. Notes: T_0 = No nitrogen and phosphorus fertilizer; T_1 = Optimized nitrogen and phosphorus fertilizer; T_2 = Excessive nitrogen fertilizer; T_3 = Excessive phosphorus fertilizer.

Fig. 3. Venn diagram depicts bacterial (A) and fungal (B) operational taxonomic units (OTUs) that were shared or unique for T_0 , T_1 , T_2 , and T_3 . Notes: T_0 = No nitrogen and phosphorus fertilizer; T_1 = Optimized nitrogen and phosphorus fertilizer; T_2 = Excessive nitrogen fertilizer; T_3 = Excessive phosphorus fertilizer.

Fig. 4. Comparison of the community structure of bacterial (A, C) and fungal (B, D) microbiota at the OTU level in different fertilization regimes using principal coordinates analysis (PCoA) and partial least squares discriminant analysis (PLS - DA) based on Bray-Curtis distance, respectively. Notes: R and P are from analysis of similarities (ANOSIM) comparing the beta diversity associated with T_0 , T_1 , T_2 , and T_3 treatments. T_0 = No nitrogen and phosphorus fertilizer; T_1 = Optimized nitrogen and phosphorus fertilizer; T_2 = Excessive nitrogen fertilizer; T_3 = Excessive phosphorus fertilizer.

Fig. 5. Average relative abundance of dominant bacterial (A) and fungal (B) phyla ($> 1.0\%$) in different fertilization regimes. The abundance is expressed as the average percentage of the targeted sequences to the total high-quality bacterial sequences of samples from triplicate plots of each fertilization regime. Notes: 'Others' refer to those identified phyla with lower than 1.0% relative abundance in all the samples. T_0 = No nitrogen and phosphorus fertilizer; T_1 = Optimized nitrogen and phosphorus fertilizer; T_2 = Excessive nitrogen fertilizer; T_3 =

Excessive phosphorus fertilizer.

Fig. 6. Redundancy analysis (RDA) of the bacterial (A) and fungal (B) communities based on edaphic factors.

Notes: T_0 = No nitrogen and phosphorus application; T_1 = Optimized nitrogen and phosphorus fertilizer; T_2 = Excessive nitrogen fertilizer; T_3 = Excessive phosphorus fertilizer. N: nitrogen; TN: total N; NO_3^- - N: nitrate N; NH_4^+ - N: ammonium N.

Fig. 7. Correlation heatmap of soil properties and relative abundances of dominant bacterial (A) and fungal (B) phyla. The color intensity indicates the correlation between soil property and relative abundance of each genus. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Notes: T_0 = No nitrogen and phosphorus fertilizer; T_1 = Optimized nitrogen and phosphorus fertilizer; T_2 = Excessive nitrogen fertilizer; T_3 = Excessive phosphorus fertilizer. SOC: soil organic carbon; N: nitrogen; TN: total N; NO_3^- - N: nitrate N; NH_4^+ - N: ammonium N; P: phosphorus; TP: Total P; K: potassium; TK: total K; AK: available K.

161x53mm (300 x 300 DPI)

160x63mm (300 x 300 DPI)

139x63mm (300 x 300 DPI)

172x128mm (300 x 300 DPI)

175x50mm (300 x 300 DPI)

175x67mm (300 x 300 DPI)

175x77mm (300 x 300 DPI)

Table 1 Amounts of fertilizer in the field

Fertilizer (kg ha ⁻¹)		T ₀	T ₁	T ₂	T ₃
Base fertilizer	Nitrogen (N)	0	144	216	144
	Phosphorus (P)	0	52	52	79
	Potassium (K)	0	79	79	79
Top-dressing fertilizer	N	0	96	144	96
	P	0	0	0	0
	K	0	119	119	119

Notes: T₀ = No nitrogen and phosphorus application; T₁ = Optimized nitrogen and phosphorus application; T₂ = Excessive nitrogen fertilizer application; T₃ = Excessive phosphorus fertilizer application.

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Table 2 Soil chemical properties as influenced by fertilization in 2018

Treatments	pH	Soil organic C (g kg ⁻¹)	Total N (g kg ⁻¹)	Total P (g kg ⁻¹)	Total K (g kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	Olsen-P (mg kg ⁻¹)	Available K (mg kg ⁻¹)
T ₀	5.97±0.08b	15.16±0.10c	2.18±0.25a	0.29±0.01d	20.31±0.73a	13.20±0.77a	35.14±6.12b	0.96±0.06d	64.99±4.62c
T ₁	6.06±0.08b	16.03±0.07b	1.84±0.28a	0.42±0.02c	20.44±0.70a	7.45±0.78b	58.95±5.00a	1.24±0.16c	93.11±6.22a
T ₂	5.94±0.08b	16.87±0.27a	2.24±0.22a	0.48±0.02b	20.28±0.89a	13.33±2.29a	62.21±2.85a	3.23±0.22a	83.25±3.32b
T ₃	6.27±0.18a	15.94±0.13b	2.04±0.13a	0.58±0.02a	21.46±0.55a	6.20±1.25b	56.12±3.76a	2.93±0.09b	91.12±4.19ab

Notes: T₀ = No nitrogen and phosphorus application; T₁ = Optimized nitrogen and phosphorus application; T₂ = Excessive nitrogen fertilizer application; T₃ = Excessive phosphorus fertilizer application. N: nitrogen; NO₃⁻ - N: nitrate N; NH₄⁺ - N: ammonium N. Values (means ± SD) with different lower-case letters in a column are significantly different at *P* < 0.05 according to Duncan's multiple range test.

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Table 3 Microbial alpha diversity as affected by fertilization in 2018

Microbe	Treatmen t	OTUs	Coverage (%)	Richness		Diversity	
				ACE	Chao 1	Shannon	Simpson ($\times 10^{-3}$)
Bacteria	T ₀	27236±	98.07±	2561±	2590±	6.47±	4.27±
		976a(b)	0.10a(b)	58a(a)	78a(a)	0.16a(a)	0.69a(b)
	T ₁	30287±	98.30±	2597±	2607±	6.30±	5.90±
		1226a(b)	0.08a(b)	210a(a)	195a(a)	0.31a(a)	0.25a(b)
	T ₂	29809±	98.25±	2679±	2666±	6.55±	3.94±
		3543a(b)	0.27a(b)	67a(a)	69a(a)	0.10a(a)	0.26a(b)
	T ₃	29433±	98.30±	2565±	2561±	6.52±	3.57±
		2107a(b)	0.19a(b)	135a(a)	125a(a)	0.17a(a)	0.62a(b)
Fungi	T ₀	50797±	99.96±	206±	208±	3.15±	95.70±
		9172a(a)	0.02a(a)	37a(b)	37a(b)	0.47a(b)	35.50a(a)
	T ₁	47089±	99.95±	212±	218±	3.04±	113.60±
		6486a(a)	0.01a(a)	55a(b)	46a(b)	0.56a(b)	59.82a(a)
	T ₂	57027±	99.95±	229±	235±	3.08±	102.68±
		7278a(a)	0.03a(a)	56a(b)	73a(b)	0.09a(b)	28.48a(a)
	T ₃	53336±	99.95±	242±	243±	3.27±	86.95±
		8656a(a)	0.02a(a)	35a(b)	37a(b)	0.09a(b)	21.83a(a)

Notes: T₀ = No nitrogen and phosphorus application; T₁ = Optimized nitrogen and phosphorus application; T₂ = Excessive nitrogen fertilizer application; T₃ = Excessive phosphorus fertilizer application. Operational taxonomic units (OTUs); Abundance-based coverage estimators (ACE). Values (means ± SD) with different lower-case letters inside and outside the parentheses in a column are significantly different between microbes or treatments, respectively, at $P < 0.05$ according to Duncan's multiple range test.