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Combined Application of Organic and Inorganic Nitrogen Fertilizers Affects Soil Prokaryotic Communities Compositions

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Abstract: As a fundamental part of the soil ecosystem, prokaryotes are involved in the preservation of soil functions. However, little is known of how the combined application of long-term organic and inorganic nitrogen fertilizer affects the prokaryotic communities' dynamics at a paddy field. A long-term positioning experiment initiated in 2013 with four treatments (NO: no N fertilizer, CN: 100% urea N with no organic fertilizer, PM: 80% urea N plus 20% N with pig manure, CM: 80% urea N plus 20% N with compost) were applied to detect the differential responses of soil physicochemical properties, and prokaryotic community structure and composition in different fertilization regimes. The results indicated that the long-term combined application of organic and inorganic nitrogen fertilizers altered the physicochemical properties to some extent and, simultaneously, established unique prokaryotic communities. In detail, the treatment of PM and CM significantly increased the content of soil organic carbon (SOC) and total nitrogen (TN) compared to NO. Moreover, a total of 31 indicator taxa were screened across the four treatments by LDA Effect Size (LEfSe) analysis following the principle of the greatest differences, which suggests that these indicator taxa were more sensitive to the fertilization. This research suggested that the combined application of long-term organic and inorganic nitrogen fertilizers not only contributed to the soil's physicochemical properties but also changed the prokaryotic community composition.

Keywords: fertilization regimes; soil physicochemical properties; prokaryotic communities; indicator taxa

1. Introduction

Among all agronomic practices, the application of inorganic fertilizers is regarded as the most active method to improve soil fertility and crop productivity [1]. A large amount of inorganic fertilizers has been applied to increase the worldwide crop yield [2]. However, the long-term unreasonable application of chemical fertilizers has resulted in serious adverse effects on the physicochemical properties of soil, such as the degradation of soil organic carbon (SOC) and soil acidification [3,4]. Moreover, severe environment pollution, induced by the excessive application of inorganic fertilizers, has aroused extensive attention [5]. Consequently, effective and environmentally-friendly fertilization regimes should be strongly advocated. Organic fertilizers are rich in nutrients mainly derived from animal manure and crop straws, which are agricultural waste [6,7]. It has been demonstrated that soil's physicochemical properties can be modified by organic fertilizer as a result of its comprehensive nutrients [8,9]. Moreover, the fertilizer efficiency of organic factors is more lasting when compared with

inorganic fertilizers [10–12]. Therefore, the application of organic fertilizers is better for the nutrient recycling of soil and the reduction of environmental pollution [13]. Unfortunately, the nutrient release of organic fertilizers is slow and unpredictable, which restricts its prevalence [14,15]. Considering the properties of chemical fertilizers and organic fertilizers, the combined application of chemical fertilizers and organic fertilizers would be a preferable strategy for nutrient management and environmental protection for soil in comparison with the application of either of them alone [16–18].

Although the productivity of soil is mainly caused by the high levels of nutrients available to crops, soil microorganisms also have a great effect on soil quality and, consequently, influence soil productivity [19,20]. As the most diverse soil microbial population, prokaryotes are involved in the process of organic decomposition, humus formation, and nutrient cycling, which are significantly influenced by fertilization regimes [21,22]. For instance, nitrifying bacteria are responsible for the process of oxidation from ammonia (NH_3) to nitrite (NO_2^-), and then from NO_2^- to nitrate (NO_3^-) under aerobic conditions, which can be absorbed by crops [23,24]. Accordingly, a pellucid explanation for the shifts in soil's prokaryotic communities under different fertilization regimes would contribute to a better understanding of the status of soil nutrient cycling.

The studies on soil microbiology mainly focus on the improvements and breakthroughs in research techniques. The method of classical isolation culture and plate counting is regarded as a milestone in soil microbial research. However, this method cannot reflect the abundance of soil microbial communities and their function [25]. The method of soil microbial biomass analysis that arose in the 1970s, which could effectively quantify the microbial turnover process of nutrients such as soil carbon, nitrogen, and phosphorus, promoted further research on the biochemical process of soil microbiology [26]. With the development of biological technology, molecular techniques such as polyacrylamide gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), clone libraries, and DNA sequencing were applied to the research of soil microorganisms in the late 1990s. Nevertheless, these methods mainly concentrated on the dominant group that can be cultivated, which makes it difficult to comprehensively explore the diversity of soil microbes [27–29]. After this, the method of high-throughput sequencing was used to study the divergence of the soil microbial community, which has been proved to be a better way to assess soil microbial ecology [30]. Notably, Illumina MiSeq sequencing, according to 16S rRNA gene libraries, was widely used to explore soil prokaryotic communities involving long-term fertilization regimes [31–33]. For example, it has been demonstrated that the application of long-term organic fertilizers would enhance the diversity of soil prokaryotic communities, especially the Gram positive microorganisms [34]. Additionally, the effects of different organic fertilizers on soil prokaryotic communities varied under distinct planting conditions [35]. However, there is less research on prokaryotic communities' dynamics of tidal sand soil, which develops from river alluvial or coastal sediments after planting crops.

In this research, a field experiment, lasting for six consecutive years with four different fertilization regimes under tidal sand soil in a double-rice cropping rotation system, was established to explore the influences of different organic fertilizers on the structure and composition of soil prokaryotic communities. The purposes of this study were: (i) to examine the changes in soil's physicochemical properties and prokaryotic community diversity under different fertilizer regimes, (ii) to detect the differences in the relative abundances of dominant phyla under each treatment, and (iii) to identify the indicator taxa under different prokaryotic taxonomic levels by LDA Effect Size (LEfSe) analysis.

2. Materials and Methods

2.1. Study Site

The experiment was carried out at Yanxi Town, Liuyang City, Hunan Province, China (113.82 E, 28.33 N) with a subtropical monsoon humid climate. The mean annual temperature was 17.5 °C and the annual average precipitation was 1562 mm. The initial soil properties of the study site are shown in Table 1.

Table 1. The initial soil properties of the studied site.

pH	Soil Organic Carbon (SOC) (g/kg)	Total N (TN) (g/kg)	Alkali-Hydrolyzable Nitrogen (AN) (mg/kg)	Total P (TP) (g/kg)	Available P (AP) (mg/kg)	Total K (TK) (g/kg)	Available K (AK) (mg/kg)
5.61	16.62	1.21	48.93	0.54	21.25	11.51	155.7

2.2. Experimental Design

The experiment was designed randomly from April 2013 to 2019 under a double-rice cropping system with four treatments and three replicates: no nitrogen fertilizer (NO), 100% urea nitrogen with no organic fertilizer (CN), 80% urea nitrogen plus 20% nitrogen with pig manure (PM), and 80% urea nitrogen plus 20% nitrogen with compost (CM). The TN applied to each treatment, except NO, was 180 kg hm^{-2} (pure N). All treatments received 105 kg hm^{-2} of K_2O and 60 kg hm^{-2} of P_2O_5 each growing season. The amount of organic materials applied in the treatments of PM and CM was based on the total amount of nitrogen contained in the organic materials. The application of phosphate fertilizers and potassium fertilizers was partially supplemented if the content of K_2O and P_2O_5 in the organic fertilizers was not enough. The physical and chemical properties of the organic materials are shown in Table 2. In this experiment, organic fertilizers and phosphate fertilizers were used as a basal fertilizer, while 60% of the inorganic nitrogen and potassium fertilizers were used as base fertilizers and 40% were used as top dressing at the tillering stage. Each plot was 4.0 × 5.0 m, which covers an area of 20 m^2. Moreover, all of the plots were separated by a cement ridge (20 × 25 cm) to prevent interactions between each plot. The crop managements of all the plots, including pests, diseases, and weeds, were coincident.

Table 2. Physical and chemical properties of the organic materials.

Organic Material	pH	N (%)	P_2O_5 (%)	K_2O (%)	C (%)	Moisture Content (%)
PM	8.38	0.23	0.23	0.13	13.49	68.7
CM	8.28	0.83	1.07	0.75	30.05	32.1

Nutrient content (%) are based on a fresh weight basis.

2.3. Sample Collection

Soil samples were collected in March 2019, before the early rice planting, to avoid interference from rice and recent fertilization. For each plot, soil was gathered from five sites, which were selected randomly except for the two edge rows to avoid the potential edge effects at 0–10 cm in depth, and, subsequently, formed a composite sample. All the samples were transmitted to the lab immediately on ice and split into two portions: one was stored at −80 °C for prokaryotic analysis and the other was stored for soil physicochemical properties analysis after being air-dried.

2.4. Soil Properties Analysis

The soil properties index measured in this research included SOC, TN, TP, pH, and SOC/TN, following the description of Bao [36]. In brief, the vitriol acid–potassium dichromate oxidation method was used to determine the level of SOC. Soil pH was measured by a pH meter (FE20-FiveEasyTM pH, Germany). TN was extracted with 98% sulfuric acid and TP was extracted with 98% sulfuric acid and perchloric acid, and then a continuous flow analyzer (AA3, Bran + Luebbe, Hamburg, Germany) was used to measure extracting solution.

2.5. Microbial Analysis

PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) was used to extract the soil DNA from 0.5 g soil from each sample, which was stored at −80 °C. Next, 1% agarose gels were used to check the quality of the extracted soil DNA. The primers 515 F:

5′-barcode-GTGCCAGCMGCCGCGGTAA and 806 R: 5′-barcode-GGACTACHVGGGTWTCTAAT were used to amplify the V4 hypervariable region of 16S rRNA. The total volume of the polymerase chain reaction (PCR) system was 25 μL, including 30 ng template DNA, 1 μL Forward Primer (5 μmol/L) and Reverse Primer (5 μmol/L), respectively, 12.5 μL 2× Taq Plus Master Mi, 3 μL BSA (2ng/μL), and 7.5 μL ddH_2O. The cycling parameters of PCR were 94 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 50 °C for 30 s, 72 °C for 60 s, and 72 °C for 7 min. Three PCR products of each sample were gathered together to reduce the biases of a reaction-level PCR. Then, a QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) was used to purify the PCR products, which were further sequenced based on the Miseq platform at Allwegene Company (Beijing, China). The raw sequences were quality filtered using 2.6 (CU, Fort Collins, CO, USA), based on the consideration described by Edgar and Cole [37,38]. The retained sequences were analyzed by USEARCH 7,1 (Joint Genome Institute MBL, Woods Hole Cornell University, CNRS, France) to generate the operational taxonomic units (OTUs) table according to 97% similarity after the chimeras were removed. The representative sequences of OTUs were classified against the Silva 16S rRNA database, based on a confidence threshold of 70% [39].

2.6. Analysis of Data

Statistical Program for Social Sciences (SPSS 20.0 Inc., Chicago, IL, USA) was applied for data statistic and charts preparing. One-way analysis of variance (ANOVA) was carried out for differences analysis and a Duncan comparison test was performed for multiple comparisons. Rarefaction was conducted through Mothur v.1.30.1 (University of Michigan, Ann Arbor, MI, USA) to study the sequencing depth of the soil samples. Nonparametric Shannon diversity index (Shannon), the richness of the Chao1 estimator (Chao1), and Good's nonparametric coverage estimator (Coverage), according to the OTU dataset, were also calculated by Mothur software. Beta diversity was analyzed by nonmetric multidimensional scaling (NMDS) based on unweighted UniFrac phylogenetic distance metrics at the OTU level with the vegan of R. Linear discriminant analysis effect size (LEfSe), according to the Kruskal–Wallis (KW) sum-rank testing. This was conducted to select the significantly different species, which were defined as the indicator taxa of prokaryotic taxa among groups. The effect size of each indicator taxa were evaluated by linear discriminant analysis (LDA). [2].

3. Results

3.1. Soil Properties

As shown in Table 3, soil physicochemical properties were modified to a large extent under different long-term fertilization regimes. Specifically, the pH value significantly decreased, by 0.17, under CN treatment but increased by 0.08 in response to CM treatment relative to NO treatment. The difference between the NO and PM treatments was not significant. Furthermore, the PM and CM treatments significantly increased the SOC by 16.42% and 22.08%, respectively, compared to the NO treatment. While no significant difference was observed between the CN and NO, and PM and CN treatments. In addition, we observed that TN varied from 1.39 to 1.73 g kg^{-1} and the minimum and maximum appeared in the treatments of NO and PM, respectively. The content of TN under the PM and CM is significantly higher than under the NO and CN treatments. Comparatively, the content of TP ranged from 348.20 to 397.63 mg kg^{-1}, and the minimum and maximum appeared in the treatments of CN and PM, respectively. As for the SOC/TN, there were no significant differences among the four treatments.

Table 3. Soil properties under different treatments.

Treatment	pH	SOC (g/kg)	TN (g/kg)	TP (mg/kg)	SOC/TN
NO	6.61 ± 0.01 b	19.97 ± 0.39 c	1.39 ± 0.06 b	360.64 ± 16.03 b	14.38 ± 0.56 a
CN	6.44 ± 0.03 c	20.91 ± 1.13 bc	1.46 ± 0.07 b	348.20 ± 14.51 c	14.29 ± 0.58 a
PM	6.64 ± 0.04 ab	23.25 ± 1.58 ab	1.73 ± 0.05 a	397.63 ± 26.38 a	13.41 ± 1.18 a
CM	6.69 ± 0.03 a	24.38 ± 1.64 a	1.68 ± 0.05 a	374.12 ± 12.71 ab	14.54 ± 0.59 a

Data are presented as the Mean ± Standard Deviation (n = 3). Different letters within columns indicate significance at $p < 0.05$ according to Duncan's test. NO: no nitrogen fertilizer. CN: 100% urea nitrogen with no organic fertilizer. PM: 80% urea nitrogen plus 20% nitrogen with pig manure. CM: 80% urea nitrogen plus 20% nitrogen with compost. SOC: soil organic carbon. TN: total nitrogen. TP: total phosphorus.

3.2. Sequencing Data

A total of 1,600,337 reads were successfully elicited from all the soil samples. After filtering, 1,561,198 effective sequences were maintained, which varied from 81,893 to 239,204 for each sample (Table S1). Moreover, 12,358 OTUs, which varied from 5815 to 6628 per sample, were identified from the whole samples based on 97% similarity. Furthermore, all of the OTUs were assorted as bacteria, with 60 phyla, 161 classes, 231 orders, 392 families, and 617 genus. A rarefaction analysis was used to study the sequencing depth and the results showed that the number of OTUs observed reached saturation, which indicated that the sequencing depth was adequate for further analysis (Figure S1). Additionally, the coverage indices for each treatment exceeded 0.95, which revealed that the sequencing capability was sufficient to occupy the majority of the prokaryotic community's features (Table 4). There were no significant differences in the OTU number, Shannon index, and Chao1 under the treatments of CN, PM, and CM, compared to the NO treatment.

Table 4. Estimated number of observed OTUs and the biodiversity, richness, and coverage of each treatment.

Treatment	Observed OTUs	Shannon	Chao1	Coverage
NO	6014.07 ± 360.68 a	10.76 ± 0.1542 a	8281.18 ± 410.84 a	0.9642 ± 0.0016 a
CN	6065.17 ± 82.41 a	10.74 ± 0.13424 a	8276.39 ± 166.56 a	0.9643 ± 0.0010 a
PM	5891.23 ± 244.51 a	10.66 ± 0.12511 a	8263.72 ± 501.33 a	0.9645 ± 0.0023 a
CM	6125.60 ± 280.43 a	10.80 ± 0.07543 a	8278.63 ± 421.21 a	0.9644 ± 0.0024 a

Data are presented as the Mean ± Standard Deviation (n = 3). Different letters within columns indicate significance at $p < 0.05$ according to Duncan's test. NO: no nitrogen fertilizer. CN: 100% urea nitrogen with no organic fertilizer. PM: 80% urea nitrogen plus 20% nitrogen with pig manure. CM: 80% urea nitrogen plus 20% nitrogen with compost. Observed OTUs, observed operational taxonomic units. Shannon, nonparametric Shannon diversity index. Chao1, richness of the Chao1 estimator. Coverage, Good's nonparametric coverage estimator.

3.3. Prokaryotic Community Structures and Compositions

According to the unweighted UniFrac phylogenetic distance, nonmetric multidimensional scaling (NMDS) was employed to detect the beta diversity of the prokaryotic community structures. The results showed that the soil prokaryotic communities varied significantly among the different fertilization treatments (Figure 1). Samples within the same treatment clustered tightly together and differentiated among treatments, with the exception of one of the sample in the NO treatment, which suggested that samples from the same treatment have a high similarity and samples from different treatments have high variation. In detail, the cluster of CM grouped apart from the other three clusters along the first components (NMDS1). Moreover, the cluster CN, NO, and PM separated from each other along the second components (NMDS2). Based on cluster positioning in the nonmetric multidimensional scaling (NDMS) space, the greatest difference was between the NO and CN treatments shown in Figure 1. Furthermore, PM and CM treatment clusters were situated between the NO and CN treatments, which suggests that an intermediate change in the prokaryotic community was obtained under the CM and PM treatments when compared to the NO treatment. In addition, the CM and PM treatments impacted the prokaryotic communities in an orthogonal manner when compared to the CN treatment. Moreover,

the two organic fertilizer treatments (PM and CM) were also situated apart, which indicated that the responses of the soil prokaryotic communities were distinct in different organic materials.

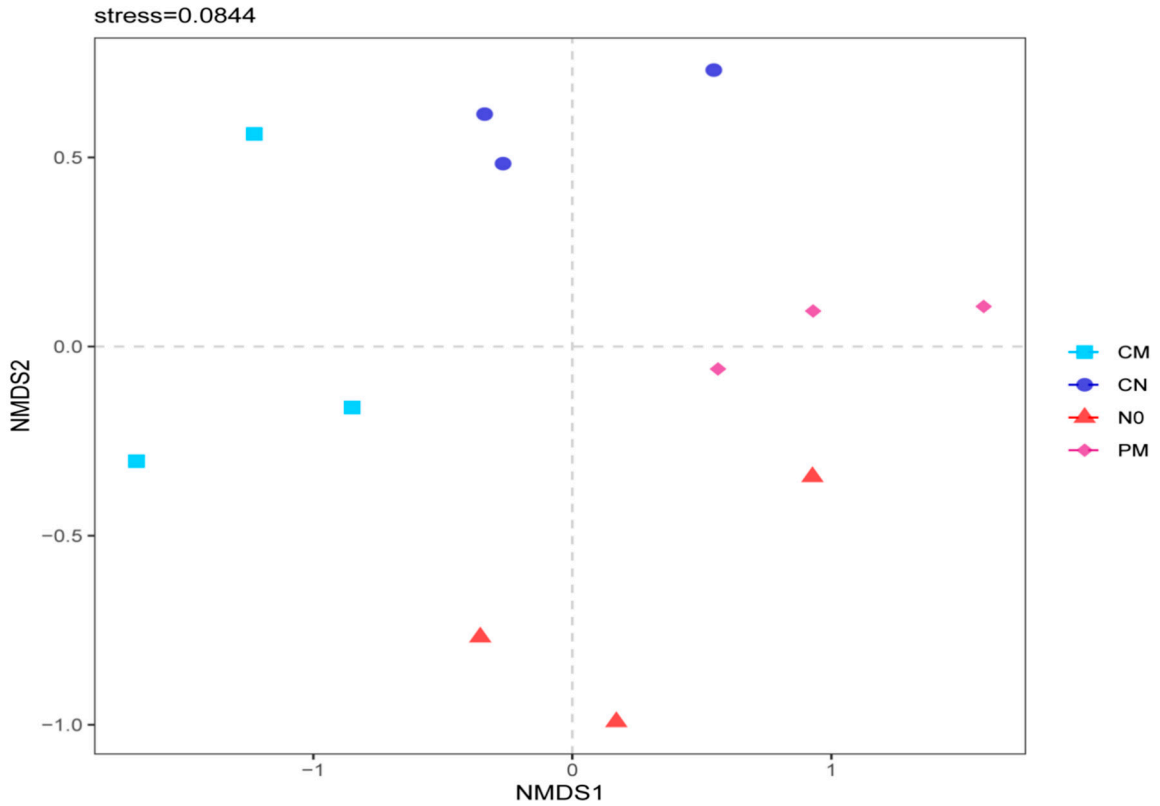

Figure 1. Beta diversity of the soil prokaryotic community of different fertilization regimes. NO: no N fertilizer. CN: 100% urea N with no organic fertilizer. PM: 80% urea N plus 20% N with pig manure. CM: 80% urea N plus 20% N with compost. Beta diversity was analyzed by nonmetric multidimensional scaling (NMDS) based on unweighted UniFrac phylogenetic distance metrics at the OTU level, and displayed in a scatter diagram.

The top five dominant phyla of each samples were *Proteobacteria*, *Acidobacteria*, *Chlorofexi*, *Actinobacteria*, and *Planctomycetes*, which occupied upward of 65% of the relative abundance of the prokaryotic communities (Figure 2). In detail, the relative abundance of *Proteobacteria* in the sample was higher than the other phyla, which was most abundant in CM treatment (38.3%) but least abundant in CN treatment (32.19%). As the second-most abundant phyla, *Acidobacteria* was most abundant in CN treatment (14.13%) but least abundant in CM treatment (11.57%). *Chlorofexi* was most abundant in PM treatment (14.32%) and least abundant in CM treatment (11.78%), whereas *Actinobacteria* was most abundant in NO treatment (7.12%) and least abundant in CN treatment (5.14%). Similarly, *Planctomycetes* was most abundant in NO treatment (6.06%) and least abundant in CN and PM treatments (4.95% and 4.875%).

To explore the differences in relative abundances of six prokaryotic phyla of the top 10 dominant bacteria, an analysis of variance (ANOVA) was applied (Figure 3). The abundances of *Proteobacteria* under the treatments of CN and PM were significantly ($p < 0.05$) decreased by 18.99% and 14.24%, when compared to the CM treatment. However, the difference in the abundances of *Proteobacteria* between CM and NO was not significant. The abundances of *Acidobacteria* increased by 8.16% under the treatment of CN compared to the treatment of no nitrogen fertilizer (NO) without a significant difference. The abundances of *Acidobacteria* decreased by 10.71% and 12.05% under the PM and CM treatments, respectively, compared to the NO treatment and the differences were not significant. The abundances of *Actinobacteria* and *Planctomycetes* were decreased by 6.85% to 27.83% and 8.43% to

19.62% under the three treatments with nitrogen fertilizer compared to the NO treatment. However, a significant difference was only observed between the CN and NO treatments for *Actinobacteria*. Regarding *Planctomycetes*, significant differences were observed in the CN and PM treatments relative to the NO treatment. The abundance of *Nitrospirae* under the CN treatment was significantly increased by 47.25% compared to the NO treatment. The abundances of *Nitrospirae* under the PM and CM treatments were increased by 20.02% and 4.28%, respectively, compared to the NO treatment, without a significant difference. There was no significant difference in the abundances of *Chlorofexi* among all of the treatments (Figure 3c).

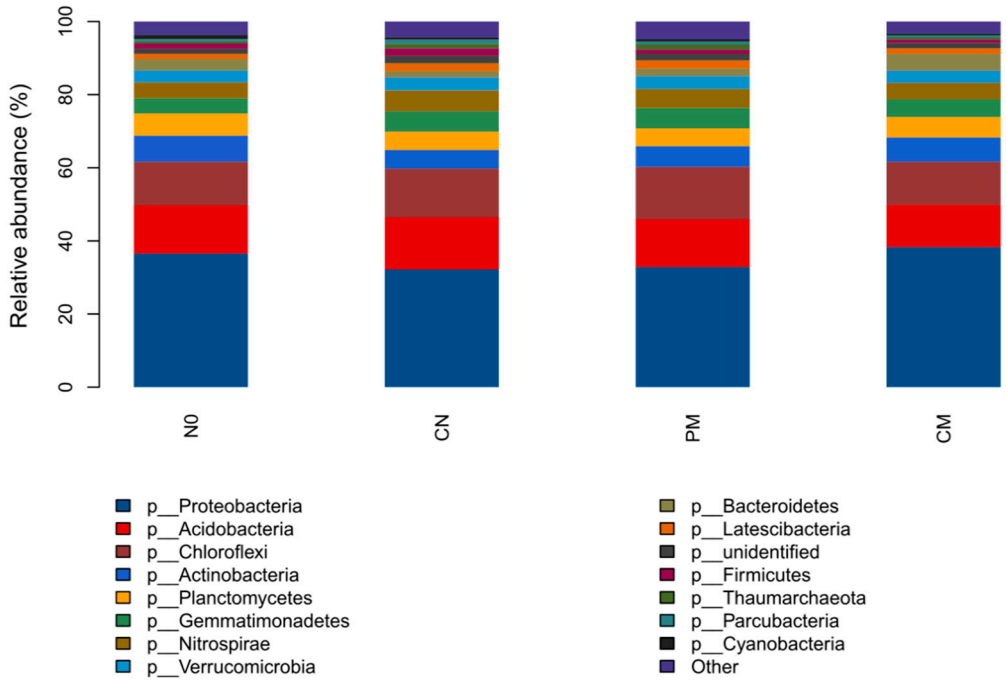

Figure 2. The relative abundances of dominant prokaryotic phyla in four kinds of organic materials amendment. Data were means of three replicates of composite samples. NO: no N fertilizer. CN: 100% urea N with no organic fertilizer. PM: 80% urea N plus 20% N with pig manure. CM: 80% urea N plus 20% N with compost.

3.4. Indicator Taxa of Different Treatments Subjected to Different Long-Term Fertilization Regimes

Linear discriminant analysis (LDA) effect size (LEfSe) was applied to identify specific prokaryotic taxa among the NO, CN, PM, and CM treatments. As shown in Figure 4, a total of 31 indicator taxa were screened across the four treatments following the principle of the greatest differences (LDA $\geq$ 3) under different taxonomic levels. The relative abundances of the kingdom *Bacteria* and order *Solirubrobacterales* were dramatically higher in CM treatment than in the other three treatments. Therefore, they were considered as the indicator taxa of CM treatment. Similarly, the class *Subgroup_22*, order *Rhodocyclales* and family *Rhodocyclaceae* were considered as the indicator taxa of CN treatment, the Phylum *Acidobacteria*, class *Alphaproteobacteria*, and *Ktedonobacteria*, order *Rhizobiales*, *Frankiales*, *Sphingomonadales*, *Hydrogenophilales*, family *Hyphomicrobiaceae*, *Rhizobiales_Incertae_Sedis*, *Acidothermaceae*, *Hydrogenophilaceae*, *Bradyrhizobiaceae*, *Sphingomonadaceae*, genus *Singulisphaera*, *Sphingomonas*, *Thiobacillus*, and *Acidothermus* as the indicator taxa of NO treatment, kingdom *Archaea*, class *SBR2076*, *South_African_Gold_Mine_Gp_1SAGMCG_1* and *Subgroup_2*, order *Ktedonobacterales*, phylum *Latescibacteria*, genus *Candidatus_Nitrosotalea*, and *Rhizomicrobium* as the indicator taxa of PM treatment.

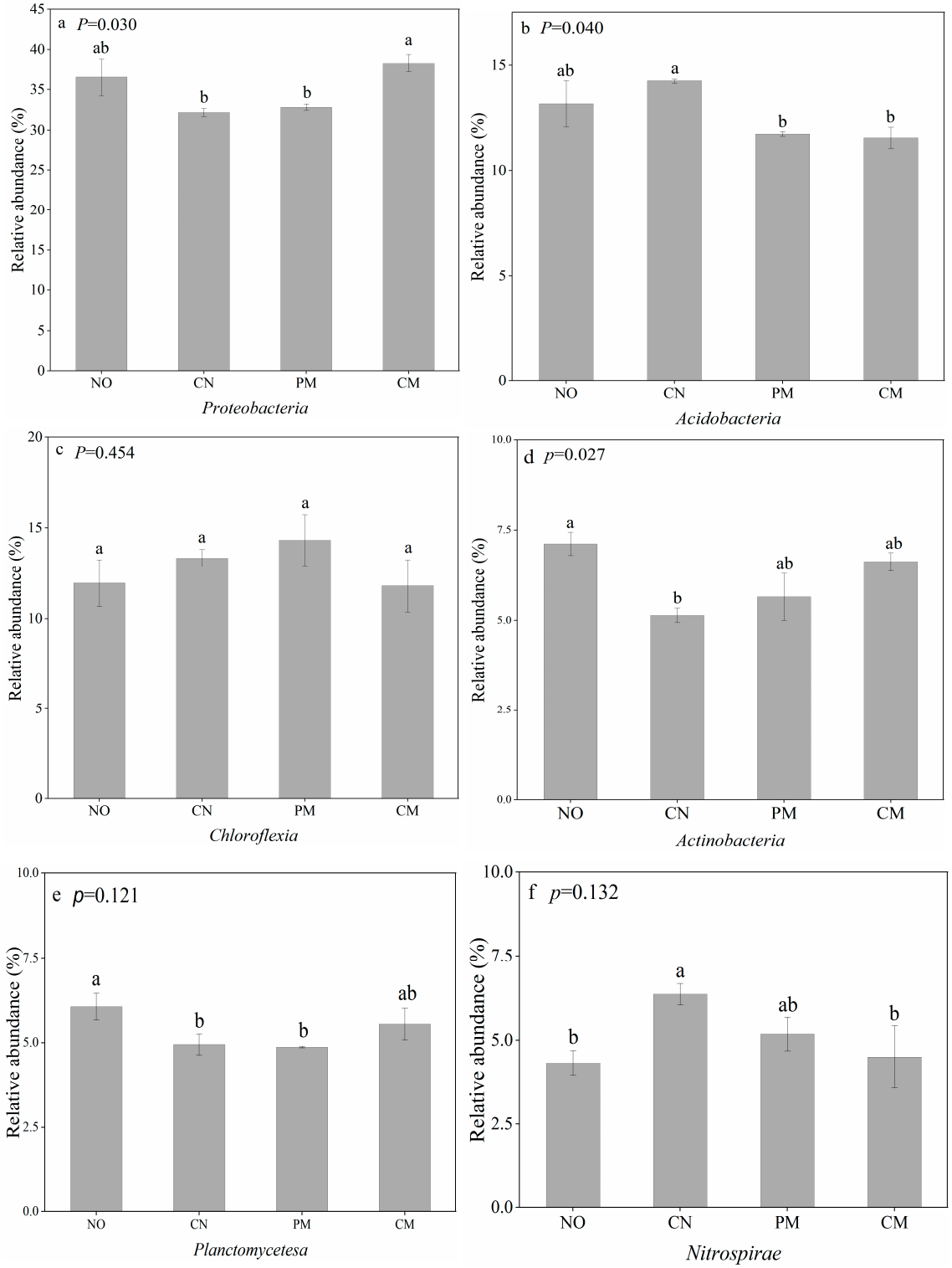

Figure 3. Comparison of the relative abundances of six bacteria phyla of the top 10 dominant bacteria phyla across treatments (*Proteobacteria*, *Acidobacteria*, *Chlorofexi*, *Actinobacteria*, *Planctomycetes* and *Nitrospirae* for (**a**–**f**) respectively). Bars indicate the standard error (n = 3). Different letters above columns within the same species indicate significance at $p < 0.05$, according to Duncan's test. NO: no N fertilizer. CN: 100% urea N with no organic fertilizer. PM: 80% urea N plus 20% N with pig manure. CM: 80% urea N plus 20% N with compost.

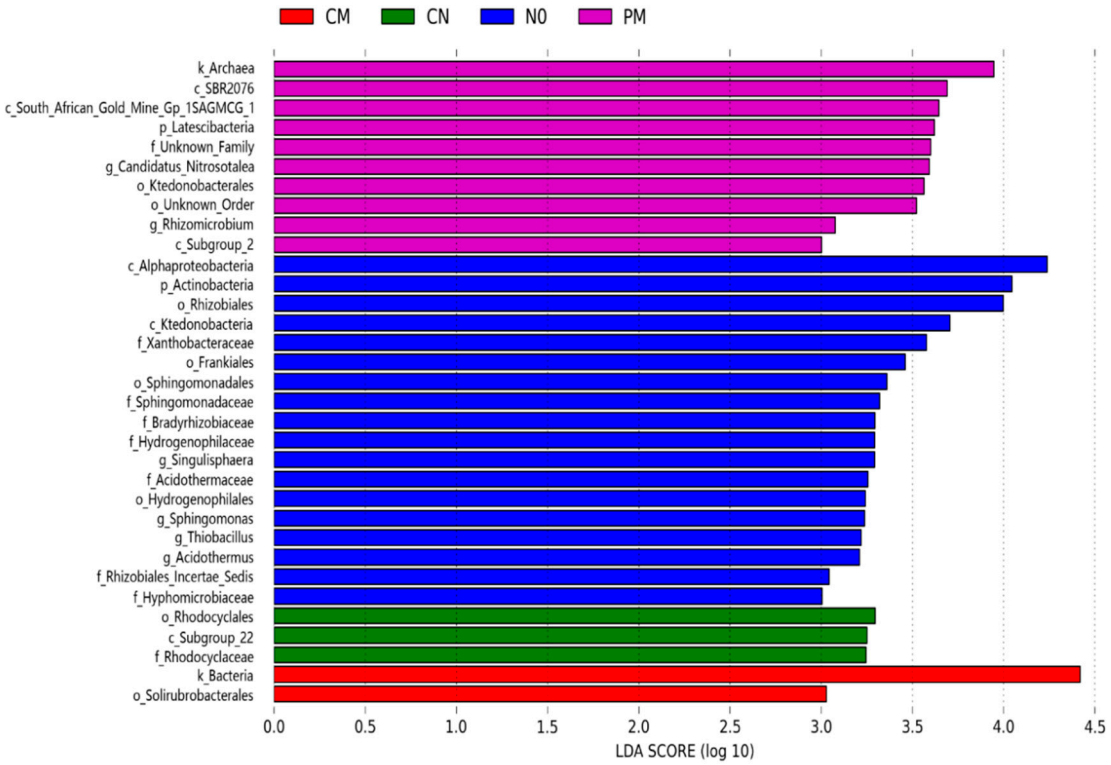

Figure 4. Taxonomic cladogram obtained from the LEfSe of 16S rDNA sequences. NO: no N fertilizer. CN: 100% urea N with no organic fertilizer. PM: 80% urea N plus 20% N with pig manure. CM: 80% urea N plus 20% N with compost.

4. Discussion

This research discovered that the combined application of long-term organic and inorganic fertilizer significantly increased the soil pH value, whereas the single application of inorganic nitrogen fertilizer significantly decreased the pH value compared with NO treatment (Table 1), which was in line with previous studies [40]. There are three possible explanations for the different response mechanisms of pH to the various fertilization regimes. First, organic fertilizer application may have increased soil pH, mainly due to the liming effect of the organic matter and carbonates in organic fertilizer [41]. Second, urea may have been converted into ammonium or ammonia in the soil first and, subsequently, the pH value decreased, due to the processes of nitrification, which converts ammonium to nitrate under the treatment of only inorganic fertilizer application [42]. Third, ammonium can decrease soil pH by completing the exchange sites of the soil solid phases with base cations [43]. Additionally, soil nutrient status was modified to different degrees under different fertilization regimes in this research. Among the four treatments, organic and inorganic fertilizer combined applications (PM, CM) most clearly increased the SOC content, which coincided with the studies of Daquiado and Wei [34,44]. We may cite two mechanisms for the increased SOC using the treatments with organic fertilizers. There was a high content of organic compounds, which were easily biodegradable in the pig manure and compost compared with inorganic fertilizer [17]. In addition, organic fertilizer could promote the growth of crops, which results in an increased input of SOC to soil via crops [45]. Moreover, the results of the present study demonstrated that the combined application of long-term organic and inorganic fertilizer would improve TN and TP content (Table 2), which is in accordance with Daquiado and Zhong [34,46]. Above all, the combined application of long-term organic and inorganic fertilizer increased the nutrient status of the soil.

The biodiversity and composition of the prokaryotic community is considered to be one of the most important factors for the material circulation and energy flow of soil ecosystems, which can be affected by fertilization regimes [47,48]. In our research, there was no significant difference in the

prokaryotic community diversity between different treatments, which was consistent with the findings of Chen and Dai [1,35]. This phenomenon could be explained by the existence of prokaryotic groups, which were not sensitive to the environmental changes. However, it contrasted with the findings of Cui, who found that the combined application of long-term organic and inorganic fertilizer resulted in a significantly ($p < 0.05$) higher prokaryotic richness index, which might be explained by the longer treatment period used in the study of Cui of 34 years from 1982 to 2016 [2].

As well as biodiversity, the differences in the prokaryotic community compositions were significant among different treatments, which corresponded with previous research [38]. In our study, the top five dominant phyla across all samples were *Proteobacteria*, *Acidobacteria*, *Chlorofexi*, *Actinobacteria*, and *Planctomycetes*, which was similar to the results of two previous studies [35,49]. As the most abundant phyla of the soil bacteria, *Proteobacteria*, which were composed of gram-negative bacteria, preferred the environment with a high nutrient content demonstrated in the study of Byss [50]. There were significant ($p < 0.05$) differences in the abundance of *Proteobacteria* under CM and PM treatments, while there were no significant differences in the SOC, TN, and TP content under the two treatments, which indicated that the abundance of *Proteobacteria* was not only affected by soil nutrient content but also fertilizer type. This echoes the previous findings of Lazcano, who found that *Proteobacteria* was particularly influenced by the types of fertilizer supplied [51]. Moreover, the phenomenon that *Acidobacteria* are able to thrive in acidic soils was observed in our study, which has also been proven in the study of Lauber and Rousk [52,53]. However, there were no significant differences in the abundances of *Chlorofexi*, which may be significantly affected by the soil water content and were not sensitive to fertilization regimes [35]. It is well known that *Actinobacteria* plays an important role in the turnover of organic matter of soil because it participates in the decomposition of polymers. However, there was no significant difference in the abundances of *Actinobacteria* under the treatments of CM, PM, and CN in our study, which contrasted with the findings of previous studies that indicated that a higher SOC drove the increase in *Actinobacteria* abundance [52,53].

5. Conclusions

The combined application of long-term organic and inorganic fertilizer increased the nutrient status of soil. Specifically, the treatment of PM and CM significantly increased the SOC and TN content compared to NO. Moreover, different fertilization regimes established the unique prokaryotic communities of paddy soil. For instance, the abundances of *Proteobacteria* under the PM treatment were significantly decreased by 10.13% compared to the NO treatment, while the difference in the abundances of *Proteobacteria* between CM and NO was not significant. In addition, LEfSe indicated that a total of 31 indicator taxa were screened across the four treatments following the principle of the greatest differences under different taxonomic levels, which suggests that these prokaryotic taxa were more sensitive to fertilization. This research suggested that the combined application of long-term organic and inorganic fertilizer not only contributed to the soil nutrients but also changed the prokaryotic community's composition.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/1/132/s1. Table S1: OTUs across treatments. Figure S1: Rarefaction curves of bacteria depicting the effect of 3% dissimilarity on the number of OTUs identified in the 12 soil samples.

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